

1 **Cross-transmission of resistant**
2 **gastrointestinal nematodes between**
3 **wildlife and transhumant sheep**

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32 **ABSTRACT**

33 Wild and domestic ungulates can be infected with the same species of gastrointestinal
34 parasitic nematodes. These parasites have free-living stage in the environment that
35 contribute to the ease of transmission among different host species. In addition,
36 gastrointestinal nematodes have developed resistance to anthelmintics which is now
37 considered a major problem for the livestock sector. In a context where wild and
38 domestic ungulates share the same pastures, the maintenance and circulation of
39 resistant gastrointestinal nematodes between species have rarely been explored.

40 In the European Alps, domestic sheep are driven to the high-altitude summer pastures
41 and live in sympatry with wild ungulates for several months. In this study we
42 investigated the nemabiome of domestic sheep and Alpine ibex, *Capra ibex*, in three
43 different areas of the French Alps to evaluate parasite circulation between the two host
44 species. The Alpine ibex is a protected mountain ungulate that is phylogenetically
45 related to sheep and hosts nematode species common to sheep.

46 Using internal transcribed spacer 2 (ITS-2) nemabiome metabarcoding, we found
47 sheep and ibex share similar gastrointestinal nematodes, except for a few species,
48 such as *Marshallagia marshalli* and *Trichostrongylus axei*. This suggests that the long-
49 term co-occurrence of sheep and ibex on mountain pastures has promoted the
50 exchange of gastrointestinal nematodes between the two hosts. Based on the
51 sequencing of the isotype 1 of the beta tubulin gene, associated with benzimidazole
52 resistance, we found resistant nematodes in all sheep flocks and in all ibex populations.
53 Our results demonstrated that ibex can host and shed resistant strains before
54 transhumant sheep arrive on pastures, and thus could act as refuge or even contribute
55 to maintain resistant gastrointestinal nematodes. The relative role of ibex in the
56 maintenance and circulation of resistant strains in sheep remain to be determined.

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58 **Keywords:** ITS-2 rDNA, benzimidazole resistance; β -tubulin isotype 1; livestock;
59 nemabiome metabarcoding; wild ungulates, transhumant sheep, Alpine ibex

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Introduction

Parasites represent a large proportion of animal diversity and are key components of food webs (Hudson et al., 2006). They are also essential determinants of the health, fitness, population dynamics and community composition of their hosts (Tompkins et al., 2011). Parasites of the Nematoda phylum infect a wide range of species worldwide, including animals and plants (Blaxter and Koutsovoulos, 2015). In animals, the gastrointestinal nematode parasites are of major concern for livestock productivity and security as they can impact animal health and reduce animal production with significant economic losses (Charlier et al., 2020; Roeber et al., 2013). Ungulates are usually infected by free-living larvae of gastrointestinal nematodes when they graze pasture. Infecting larvae may survive several months in the environment depending on species and climatic conditions (O'Connor et al., 2006). Following ingestion, larvae finish their development to reach the adult stage in the digestive tract. Egg-laying occurs 2–4 weeks post infection. The duration of infection by nematodes vary depending on species but last for at least 2 months from the L3 ingestion (Deplazes et al., 2016). The larvae can arrest their development in host (hypobiosis) during harsh climatic condition, delaying the egg-laying (Deplazes et al., 2016).

To limit parasite load and its impact on livestock health, the use of anthelmintics to treat livestock against gastrointestinal nematodes is a common and cost effective practice (Vercruysse et al., 2018). Nonetheless, the repeated use of anthelmintics has led to the selection of anthelmintic-resistant strains of gastrointestinal nematodes. Resistance to several families of anthelmintics (e.g., benzimidazole, macrocyclic lactones and levamisole) has been observed, and multiple resistance is increasing (Bordes et al., 2020; Kaplan and Vidyashankar, 2012; Rose et al., 2015; Rose Vineer et al., 2020).

In particular, resistance to benzimidazoles is widespread throughout the world (Kaplan and Vidyashankar, 2012), and is particularly common on sheep farms in Europe (Papadopoulos et al., 2012; Rose Vineer et al., 2020). Contrary to other anthelmintic families, the mechanisms of resistance to benzimidazole are well known and documented (Whittaker et al., 2017). In resistant nematodes, specific mutations of the β -tubulin isotype-1 gene have been correlated with the resistance to benzimidazole in several gastrointestinal nematode species (Charlier et al., 2022). Furthermore, large-scale screening based on molecular tools is now feasible for this resistance

99 (Avramenko et al., 2019), whereas the recommended method in livestock (i.e., the
100 fecal egg count reduction test; Kaplan et al., 2023) for the diagnosis of resistance to
101 other anthelmintics requires techniques that are difficult to achieve in wildlife in remote
102 fields.

103 Some generalist gastrointestinal nematodes can infect several host species
104 (Walker and Morgan, 2014), including both domestic and wild ungulates (e.g.,
105 Beaumelle et al., 2022; Cerutti et al., 2010). The transmission of gastrointestinal
106 nematodes among hosts, even if they do not simultaneously occupy the same
107 pastures, is possible thanks to their free-living infective larval stage that may active
108 several months in the environment (Carlsson et al., 2013; Fiel et al., 2012; Walker and
109 Morgan, 2014). Transmitted parasites can also include gastrointestinal nematodes
110 resistant to anthelmintics. For instance, benzimidazole-resistant nematodes have been
111 detected in free-living populations of roe deer, *Capreolus capreolus*, living in sympatry
112 with livestock (Chintoan-Uta et al., 2014; Nagy et al., 2017). To date, the role of wild
113 ungulates in the epidemiology of resistant nematodes remains to be determined, but it
114 has been suggested that wildlife may act as a reservoir of resistant nematodes for
115 livestock (Brown et al., 2022; Chintoan-Uta et al., 2014; Francis and Šlapeta, 2023;
116 Laca Megyesi et al., 2019; Walker and Morgan, 2014). However, to accurately evaluate
117 the potential role of wildlife as reservoirs for anthelmintic resistant gastrointestinal
118 nematodes, we need to investigate the presence of resistant nematodes in co-grazing
119 wild and domestic ungulates in different contexts, (i.e., different host species, different
120 landscapes, and under different climatic conditions).

121 Transhumant pastoralism is a common practice in the European Alps and consists
122 in the seasonal movement of grazing livestock from lowland areas to mountain
123 meadows in summer which provide fresh pasture for domestic ungulates, i.e., mainly
124 sheep, but also cows or goats (Biber, 2010). These mountainous areas are inhabited
125 year-round by wild ungulates, particularly those living at high altitude in the European
126 Alps, like Alpine ibex (*Capra ibex*), or Northern chamois (*Rupicapra rupicapra*). While
127 wild ungulates tend to avoid domestic herds spatially or temporarily during the summer
128 (Acevedo et al., 2008), certain factors may contribute to the use of the same pastures
129 by both groups.

130 Spatial segregation between wild and domestic ungulates is usually observed once
131 livestock arrive on pasture (Brivio et al., 2022; Ryser-Degiorgis et al., 2002). Livestock
132 are generally released onto the best grazing areas during the summer season

133 (Chirichella et al., 2014; Richomme et al., 2006; Ryser-Degiorgis et al., 2002). Prior to
134 their arrival, mountain ungulates have been observed to preferentially use the same
135 grazing areas both before and after use by livestock (Brivio et al., 2022; Ryser-
136 Degiorgis et al., 2002). The presence of wild and domestic ungulates in attracting
137 zones such as salt licks, even if not simultaneous, offers good opportunities for parasite
138 transmission, and these areas are therefore considered hotspots for parasite infection
139 (Richomme et al., 2006; Ryser-Degiorgis et al., 2002; Utaaker et al., 2023).

140 Consequently, transhumant pastoralism represents a risk for pathogen
141 transmission between wild and domestic ungulates in mountain areas (Rossi et al.,
142 2019). Pathogen exchange at the interface of wild and domestic ungulates have
143 already been well documented. The Alpine ibex has been identified as the wildlife
144 reservoir of brucellosis (*Brucella melitensis*) which was transmitted to cattle and
145 humans in the Bargy massif in northern French Alps (Marchand et al., 2017). In
146 addition, sheep have been confirmed as the domestic reservoir of Border disease,
147 which induced a major viral outbreak in Southern chamois (*Rupicapra pyrenaica*)
148 populations in the Pyrenees (Luzzago et al., 2016). The transmission of
149 gastrointestinal nematodes has already been described between wild ungulates and
150 transhumant domestic ungulates in mountainous areas (Cerutti et al., 2010; Citterio et
151 al., 2006; Khanyari et al., 2022; Zaffaroni et al., 2000). However, no study has yet
152 investigated the transmission of anthelmintic-resistant nematodes in a transhumant
153 pastoral system.

154 In this study, we investigated the community of gastrointestinal nematodes infecting
155 Alpine ibex and domestic sheep (*Ovis aries*) and the prevalence of resistance to
156 benzimidazole, in three different regions of the French Alps. The Alpine ibex was close
157 to extinction at the beginning of the 19th century but the reinforcement of its populations
158 by several reintroductions in different part of the Alps has increased the species' overall
159 abundance and range (Brambilla et al., 2022). Today, the Alpine ibex species is
160 estimated at 52 000 individuals in Europe (Brambilla et al., 2020).

161 Ibex usually host species-specific gastrointestinal nematodes (Walker and Morgan,
162 2014) but they may also be exposed to generalist nematodes deposited by other
163 related ungulates species, that live in the same area at least part of the year, being
164 wild (i.e., Northern chamois, *Rupicapra rupicapra*) or domestic (e.g., sheep).
165 Furthermore, anthelmintic treatments are frequently applied to livestock by farmers
166 with the aim of reducing the parasite load and hence reducing the diversity of

167 nematodes in sheep. Therefore, we expected the nemabiome to be highly
168 differentiated between the two species in the three mountain areas, with a higher
169 nematode diversity in ibex compared to sheep (H1). We expected sheep to host
170 benzimidazole-resistant strains of gastrointestinal nematode, in line with the general
171 pattern observed for sheep in France (Papadopoulos et al., 2012; Rose Vineer et al.,
172 2020). With the implementation of reintroduction programs in the second half of the
173 20th century, ibex have colonized pastures traditionally grazed by sheep. We therefore
174 expected that ibex will also host benzimidazole-resistant gastrointestinal nematodes
175 but to a lesser extent, as resistance do not represent a selective advantage for
176 nematodes in the ibex environment (Hahnel et al., 2018) (H2). Because there are very
177 few documented ibex dispersal events among the 3 ibex populations ((Brambilla,
178 2020), R. Papet, C. Toïgo and E. Vannard, personal communication), we predicted
179 genetic differences among nematodes species/community or strains (ASV: Amplicon
180 sequence variant) among the populations of ibex due to genetic drift (H3).

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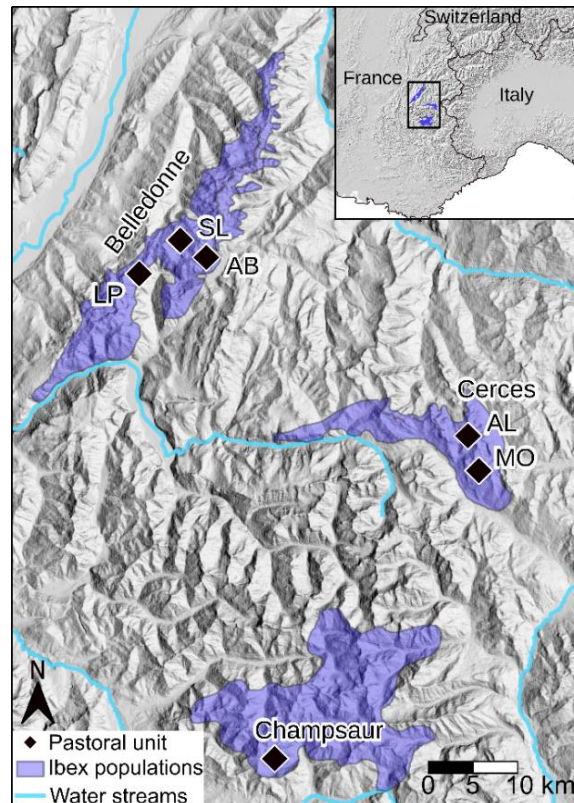
Materials and Methods

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Study area

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186 Samples of sheep and ibex feces were collected in the French Alps in 3 different
187 mountain areas (Figure 1). The Belledonne mountain is located in the western part of
188 the Alps in southeast France. The Cerces and Champsaur mountains are in the north
189 and the south parts of the Ecrins National Park, respectively (Figure 1).



190

191 **Figure 1** : Sampling locations in the French Alps where sheep and ibex feces were
 192 collected. Pastoral unit: area where both ibex and sheep have been sampled. Cerces :
 193 AL: Aiguillette de Lauzet, and MO: Montagne de l’Oule ; Belledonne : AB: Ane Buyant,
 194 LP : La Pesée and SL : Sept Laux.

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196 The 3 study areas are characterized by the presence of steep slopes, high
 197 peaks (>2500m) and agropastoral activities. Climatic conditions are harsh in these
 198 mountains with a mean temperature in winter (December-March) 2015-2019 of 0°C in
 199 Belledonne (alt:1785m), 1°C in Champsaur (alt:1620m) and 1.5°C in Cerces
 200 (alt:1553m). During summer (June-September), the mean temperature is 13°C in
 201 Belledonne, 14°C in Champsaur and 16°C in the Cerces (Réseau d’Observation Météo
 202 du Massif Alpin; www.romma.fr). Champsaur is the southern study area and has a
 203 Mediterranean influence. Consequently, rainfall is less important in this area compared
 204 to Cerces and Belledonne. The vegetation is distributed along an elevation gradient
 205 from coniferous woodland (*Abies alba* and *Picea abies* in Belledonne and *Larix*
 206 *decidua* and *Pinus sylvestris* in Cerces and Champsaur) in the lower range of ibex, to
 207 a landscape dominated by heathland with *Rhododendron ferrugineum*, *Vaccinium* spp.
 208 and *Juniperus communis*, and grassland (*Carex* spp. *Festuca* spp.) above the tree line
 209 (Ozenda, 1985).

210 The ibex populations were established in Belledonne, Cerces and Champsaur,
 211 in 1983 with the introduction of 20 ibex, in 1959-1961 with the introduction of 6 ibex
 212 and in 1994-1995 with the introduction of 30 ibex, respectively. Traditional pastoral
 213 activity is practiced in all massifs, where sheep flocks arrive early summer to graze
 214 mountain pastures coming from the plain on foot or by truck.

215 In the Belledonne mountain, the distribution of ibex range between 630m and
 216 2860m on 200km². Populations size is estimated to 800. The size of the herds are 750
 217 ewes followed by their lambs in La Pesée, 900 ewes in Sept Laux, and 1600 ewes in
 218 Ane Buyant. A dozen rams are also present within the La Pesée and Sept Laux herds,
 219 as well as some goats in La Pesée. Each sheep herd belongs to one farmer while
 220 several farmers grouped their sheep herds in Cerces and Champsaur (table 1).

221 The ibex population located in the Cerces mountain is estimated to 320
 222 individuals and occupy an area of 120km², between 1410m and 3100m. In Cerces, the
 223 herd located in the West (Aiguillette du Lauzet) included 3 breeding farms for a total of
 224 800 sheep and the herd located in the East (Montagne de l'Oule) included 4 breeding
 225 farms for a total of 940 sheep (table 1).

226 In the Champsaur mountain, the distribution of ibex range between 1320m and
 227 3550m on 280km². Population size is estimated to 420 individuals. In Champsaur, the
 228 herd included 4 breeding farms for a total of 1070 sheep and 5 goats (table 1).

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 230 **Table 1 : Descriptive data on study sites.** AL: Aiguillette de Lauzet, and MO: Montagne
 231 de l'Oule ; Belledonne : AB: Ane Buyant, LP : La Pesée and SL : Sept Laux.

	Area (km ²)	Altitude (m)	Number of introduced ibex	Ibex Population size	Domestic flock size	Climate
Belledonne mountain	200	630-2860	20	800	AB: 1600 LP: 750 SL: 900	Mountain climate
Cerces mountain	120	1410-3100	6	320	AL: 800 MO: 940	Mountain climate
Champsaur mountain	280	1320-3550	30	420	1070	Mountain climate with Mediterranean influence

233 *Sample collection*

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235 We collected sheep feces for 15 days after arrival on pasture to ensure that we
236 collected nematode species representative of sheep at the time of arrival on pastures
237 and not those ingested secondarily on alpine pastures. Similarly, ibex feces were
238 collected prior to the arrival sheep and until 15 days after arrival to ensure that the
239 nematode community was not influenced by the arrival of domestic livestock. Fresh
240 ibex feces were mostly collected directly on the ground and, in Belledonne, also during
241 captures as part of the long-term monitoring program conducted by the French Office
242 for Biodiversity. Ibex feces were collected within each of the pastoral units in which we
243 collected sheep feces. Where possible, feces were collected immediately after
244 observation of ibex to avoid collecting of feces from the same individual. Feces from
245 all age groups were collected. Samples were stored in plastic bags, sealed after air
246 removal, and analyzed within 48h upon receipt in the parasitology laboratory of the
247 National Veterinary School of Lyon (ENVL, Marcy-l'Étoile, France) or up to a maximum
248 of 15 days after field collection (mean: 2.5 [min: 0 - max: 15] days). In total, we sampled
249 167 fecal samples from ibex and 90 fecal samples from 6 sheep herds, distributed over
250 6 pastoral units, i.e., Aiguillette du Lauzet, Montagne de l'Oule, Champsaur, Ane
251 Buyant, La Pesée and Sept Laux (Table 2, Figure S1). In Belledonne, 21 samples were
252 collected in 2018 following the sampling strategy of 2019. We controlled that year of
253 sampling did not result in drastic change of nemabiome in ibex (figure S6) and included
254 those samples in analyses.

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265 **Table 2** : Location, period and number of samples collected in the French alps.
 266 Coproscopic results of the gastro-intestinal (GI) nematodes (strongyles) in eggs per
 267 gram (epg) of faeces are also reported (median[min-max]). Cerces : AL: Aiguillette de
 268 Lauzet, and MO: Montagne de l'Oule ; Belledonne : AB: Ane Buyant, LP : La Pesée
 269 and SL : Sept Laux.

	Sampling date	N	GI nematodes epg
Cerces mountain			
Sheep	June 2019	AL: 15	AL: 7.5 [0-60]
		MO: 15	MO: 7.5 [0-225]
Ibex	AL: May-June 2019	AL: 29	AL: 7.5 [0-30]
	MO: May 2019	MO: 18	MO: 7.5 [0-60]
Champsaur mountain			
Sheep	June 2019	15	7.5 [0-30]
Ibex	May 2019	40	7.5 [7.5-105]
Belledonne mountain			
Sheep	July 2019	AB: 15	AB: 30 [0-90]
		LP: 15	LP: 7.5 [0-165]
		SL: 15	SL: 0 [0-30]
Ibex	July 2018 and May-June 2019	80	15 [0-525]

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271 *Parasitological analyses*

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273 The number of gastro-intestinal nematodes eggs per gram of feces (epg) was
 274 counted following a modified McMaster protocol (Raynaud et al., 1970) with a solution
 275 of zinc sulphate (ZnSO₄, density = 1.36, 1/15 dilution). The eggs were counted on a
 276 McMaster slide with two chambers (theoretical sensitivity of 15 eggs per gram of faeces
 277 [epg]). We also checked for the presence of low abundant parasite propagules with a
 278 14 mL tube filled with the remaining solution and covered with a coverslip before
 279 centrifugation (5 min at 1200 rpm) and microscopical observation ('control slide'). We
 280 attributed the value of 7.5 epg for parasites with no egg observed on the McMaster,
 281 but at least one egg observed on the control slide (for a similar procedure see
 282 Beaumelle et al, 2021).

283 In order that strongyles reach the L3 stage, coprocultures of feces were done at
 284 24 ± 1 °C during 12-15 days with regular mixing and moistening. We then collected the
 285 L3 in tape water with a Baermann apparatus. We extracted gastrointestinal nematodes

286 DNA from samples for which there were at least 20 L3 and we limited the extraction to
287 ~200 L3. DNA was extracted using extraction kit (Qiagen DNeasy® PowerSoil)
288 following the manufacturer's instruction with an elution volume of 50 µl of water. We
289 extracted twice the DNA of 30 randomly chosen samples as internal extraction controls
290 (Taberlet et al., 2018). We quantified DNA concentration for all samples using Qubit
291 2.0 fluorometer (Life Technologies) and homogenized DNA samples to a DNA
292 concentration of 1ng/µl (DNA samples were not diluted if the DNA concentration was
293 <1 ng/µl).

294

295 *High throughput sequencing analyses*

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297 To determine the nemabiome of sheep and ibex we used a modified version of
298 the protocol developed by Avramenko et al., (2015). The ITS2 region of the nuclear
299 rDNA was amplified using the primer pair NC1 (Forward - 5'-
300 ACGTCTGGTTCAGGGTTGTT-3') and NC2 (Reverse- 5'-
301 TTAGTTTCTTTTCCTCCGCT-3') with the following PCR conditions: 10µl of Applied
302 Biosystems™ Master Mix AmpliTaq Gold™ 360, 5,84µl of molecular biology grade
303 water, 0,16µl of Bovine Serum Albumin, 2µL of 5 µM mixed F and R primers form, 2
304 µL of DNA lysate. The PCR was performed under the following conditions: 10 min initial
305 denaturation at 95°C; 35 cycles of denaturation (30 s at 95°C), annealing (30 s at
306 54°C), and extension (1 min at 72°C); a final extension at 72°C for 7 min. The
307 thermocycling parameters were choose identically to Avramenko et al., (2015).

308 To detect the mutations responsible for the resistance of gastrointestinal
309 nematodes to benzimidazole, we used a modified protocol of Avramenko et al., (2019).
310 Using the same mix as previously described, we amplified the β-tubulin isotype 1
311 fragment comprising the codons at position 167, 198 and 200 with two pairs of primers
312 in two independent PCR. The PCR was performed under the following conditions: 10
313 min initial denaturation at 95°C; 40 cycles of denaturation (30 s at 95°C), annealing (30
314 s at 65°C), and extension (30 s at 72°C); a final extension at 72°C for 7 min. We
315 targeted *Teladorsagia circumcincta* and *Trichostrongylus* spp. (Forward:5'-
316 CGCATTCWCTTGGAGGAGG-3' and Reverse: 5'-
317 GTGAGYTTCAAWGTGCGGAAG-3') and *Haemonchus contortus* (Forward:5'-
318 CGCATTCYTTGGGAGGAGG-3' and Reverse: 5'-GTGAGTTTYAAGGTGCGGAAG-

319 3') with the primers described by Avramenko et al., (2019). All forward and reverse
320 primers were tagged at 5' in order that each sample had a unique combination of
321 tagged primers.

322 In all PCRs, we added positive PCR controls (i.e., *Haemonchus contortus* and
323 *Teladorsagia circumcincta* DNA extracts), negative PCR controls (distilled H₂O) and
324 negative DNA extraction controls. All samples (including controls) were tagged with
325 unique barcode identifiers to allow pooling into a single amplicon library (Taberlet et
326 al., 2018), and all samples were independently amplified 4 times to ensure reliability of
327 the sequencing. Amplifications were carried out in 96-well plates, totaling 209 ibex and
328 sheep samples, 17 PCR positive controls, 13 PCR negative controls, 7 extraction
329 negative controls, 30 DNA extraction controls, as well as 12 empty wells in each plate
330 to quantify tag jumping during PCR and sequencing steps (Figure S2; De Barba et al.,
331 2014; Taberlet et al., 2018).

332 All PCR products of the ITS2 and the two β -tubulin isotype 1 sets were purified
333 using QIAquick® Spin Columns (QIAquick® PCR Purification Kit QIAGEN) and
334 quantified using a Qubit 2.0 fluorometer (Life Technologies). Next, we pooled the 3
335 purified DNA pools (ITS2, two β -tubulin isotype 1) based on their initial concentration
336 and in proportion according to the following ratio: ITS2 50%, β -tubulin isotype 1 25%
337 for each. According to preliminary tests, we expected to achieve a sequencing depth
338 of 20 000 reads per ITS2 DNA sample and 5 000 reads per β -tubulin isotype 1 DNA
339 sample. Sequencing was performed with pair-end sequencing technology on the
340 Illumina platform (2*250 bp Miseq) at Fasteris, Geneva, Switzerland.

341

342 *Sequence analysis and taxon assignment*

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344 The sequence reads were first analyzed with the OBITOOLS package (Boyer et al.,
345 2016). Forward and reverse reads were assembled with the *alignpairedend* function,
346 and we kept only sequences with a good score of alignment ($r_{norm} > 0.8$). Sequences
347 were attributed to their samples with the *ngsfilter* function with default parameters.
348 Subsequently, assigned sequences were analyzed with the *dada2* package (Callahan
349 et al., 2016) following the pipeline available in www.nemabiome.ca. The *dada2* pipeline
350 returns Amplicon Sequence Variants (ASV) which are sequence variants differing by
351 as little as one nucleotide (Callahan et al., 2017). Following Beaumelle et al. (2021),

352 gastrointestinal nematodes were identified with four different methods of assignment-
353 databases: BLASTn (Altschul et al., 1990) based on (1) the NCBI database (Accessed:
354 November 2022), and (2) AssignTaxonomy (Callahan et al., 2016; Wang et al., 2007)
355 and (3) IDTaxa (Murali et al., 2018) based on the nematode ITS2 rDNA database 1.1.0
356 (Workentine et al., 2020). To identify the species associated with the β -tubulin
357 sequences, we used IDTaxa against the nematode β -tubulin isotype 1 DNA reference
358 sequences supplied in Avramenko et al., (2019). We chose to attribute a confidence
359 level to taxonomic identifications at the species level: high or moderate confidence if
360 three or two methods of assignment, respectively, were congruent. We also adjusted
361 the sequence filtering based on an adapted procedure of Calderón-Sanou et al. (2020).
362 We kept only ASVs present in at least 2 replicates of the same samples and removed
363 ASVs that were not assigned to the genus level for the ITS2 and to the species level
364 for β -tubulin isotype 1. We removed potential contaminants (reagent contaminants and
365 cross-contaminations) following the procedure detailed in Calderón-Sanou et al.,
366 (2020). For each sample, we summed the reads of the two replicates with the highest
367 similarity. If this similarity was lower than the mean similarity among all replicates,
368 sample was discarded. Then, we verified that the 30 DNA extraction replicates had
369 similar nemabiomes and kept one sample replicate out of two. At the end, we removed
370 samples if they had <1000 reads of ITS2 and <500 reads of β -tubulin isotype 1 (Figure
371 S3).

372

373 *Identification of non-synonymous mutations in codons 167, 198 and 200*

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375 For each nematode species, all β -tubulin isotype 1 ASVs were aligned to one of
376 the β -tubulin isotype 1 consensus sequences of the reference database (Avramenko
377 et al., 2019) using the *AlignSeqs* function of the DECIPHER package (Wright, 2016).
378 We examined each β -tubulin isotype 1 ASV at codons 167,198 and 200 to record
379 whether the codon was associated with a non-synonymous mutation. However, we
380 ignored the other polymorphic sites of the Exon.

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384 *Statistical analyses on measures of nemabiomes*

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386 To measure differences of nemabiomes among the two host species (sheep and ibex)
387 and the 4 study sites (Aiguillette de Lauzet, Montagne de l'Oule, Champsaur and
388 Belledonne), we considered two measures of diversity, i.e., the alpha diversity and the
389 beta diversity. The alpha diversity was measured with the Shannon index that
390 considers richness and evenness of communities and the beta diversity was measured
391 with the weighted UniFrac index estimated using the R phyloseq package (McMurdie
392 and Holmes, 2013). The weighted UniFrac distance is a phylogenetic distance
393 between the set of ASVs from each nemabiome weighted by the transformed
394 abundance of each ASV (Lozupone and Knight, 2005). The phylogenetic distances
395 were computed from a phylogenetic tree which was **constructed** using maximum
396 likelihood with the GTR+G+I model according to the *ModelTest* function (Posada and
397 Crandall, 1998; Schliep, 2011). The exact counts of ITS2 reads were transformed with
398 the Hellinger transformation (e.g., square root of relative frequencies) to account for
399 the high number of zeros in the community tables and to decrease the influence of rare
400 ASVs in statistical analyses (Legendre and Legendre, 2012).

401 We tested the effects of host species and site, including their interaction, on alpha
402 diversity with linear models, and on beta diversity using perMANOVA (*adonis2*, vegan
403 R package (Oksanen et al., 2020)). All possible models including the null model were
404 computed. For perMANOVA models, we used a custom function to compute Akaike's
405 information criterion corrected for small sample size (AICc) based on residual sums of
406 squares (Dyson, 2018). In a model selection approach, for both alpha and beta
407 diversity, all possible models were ranked using the AICc and we selected the model
408 with the lowest AICc value. Models with $\Delta\text{AICc} \leq 2$ were considered equivalent
409 (Burnham and Anderson, 2002), and in this case, we considered the most
410 parsimonious one, i.e., the model with the lowest degrees of freedom.

411 **As sex and age have been determined for some ibex, we also tested the effect of ibex**
412 **classes (1: adult males, 2: females or kids/yearlings) on alpha and beta diversity**
413 **following the same model selection approach, including ibex classes and sites as**
414 **explanatory variables.**

415 All analyses were carried out using R 3.6 (R Core team, 2020. [https://www.R-](https://www.R-project.org/)
416 [project.org/](https://www.R-project.org/)).

417 *Statistical analyses on measures of resistant nematode strains*

418

419 To compare the importance of benzimidazole resistance in gastrointestinal nematodes
420 between ibex and sheep and among the 3 sites, we tested if the host species, the site
421 and the nematode species influenced the relative abundance of ASVs with a resistant
422 allele. For this purpose, we used a generalized linear model with a binomial family and
423 a model selection approach such as described above.

424 We used *AlignSeqs* (Wright, 2016) to generate multi-sequence aligned β -tubulin
425 isotype 1 haplotype data. For each gastrointestinal species, we removed short ASVs,
426 e.g., ASVs with a sequence length <10% compared to the median ASV length.
427 PopART v1.7 (Leigh and Bryant, 2015) was used to draw median joining networks
428 based on the haplotypes data of each gastrointestinal nematode species.

429

430

Results

431

432 *Parasite material*

433

434 The median number of eggs of strongyles per gram of feces were lower in sheep
435 (7.5[0,148]_{95%IQR}; n = 90) than in ibex (15[0,163]_{95%IQR}; n = 167) feces (Mann–Whitney
436 U test; $W = 9043.5$, $P = 0.006$). As a result of the low level of infestation in some
437 samples, the number of L3 hatched from eggs were not sufficient ($n < 20$) for 48 ibex or
438 sheep samples. These samples were not used for subsequent genetic investigations.
439 Specifically, all samples from the Sept Laux sheep herd (n=15, Belledonne) were
440 discarded. Therefore, the nemabiome was determined based on the ITS2 for 196 (n=
441 55 sheep and n=141 ibex) out of 209 samples for which DNA was extracted (Figure
442 S1).

443

444 *Diversity of gastrointestinal nematodes in sheep and ibex*

445

446 In total, we detected 408 ASVs corresponding to 13 gastrointestinal nematode
447 species (Table 3, Figure S4). Eight ASVs were assigned to the genus level (i.e.,
448 *Marshallagia* spp., *Nematodirus* spp. and *Trichostrongylus* spp.) due to non-identical
449 assignation among taxonomic methods. An ASV corresponding to the lungworm

450 *Cystocaulus ocreatus* was discarded for the statistical analyses because we only focus
451 on gastrointestinal nematodes.

452 *Teladorsagia circumcincta* was the most prevalent nematode species and was
453 detected in 85% of samples (90%, n=127/141 ibex and 71%, n=39/55 sheep), followed
454 by *Trichostrongylus vitrinus*, 63% (73%, n=103/141 ibex and 36%, n=20/55 sheep) and
455 *Haemonchus contortus*, 56% (70%, n=98/141 ibex and 22%, n=12/55 sheep) (Table
456 3, Figure S4). *Nematodirus* spp. and *Ostertagia leptospicularis* were the rarest species
457 and were detected in only 2 and 1 sample, respectively and with a very low relative
458 frequency (<0.1%).

459 The parasite of the genus *Nematodirus* were not considered for the following results
460 as our coproculture protocol is not the most appropriate for these parasites as some
461 species need more than 2 weeks to reach the L3 stage and need to be exposed to cold
462 temperatures before coproculture, which can be deleterious for other strongyle species
463 such as *Haemonchus contortus* (van Wyk and Mayhew, 2013).

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483 **Table 3** : Number of ASVs, reads and samples for each nematode taxon, including the
 484 results from the ITS2 (nemabiome) and the β -tubulin isotype 1 (resistance to
 485 benzimidazole). N= number (and percentage) of samples in which a taxon was
 486 detected. Host species and study sites are mixed here.

	ITS2			β -tubulin isotype 1		
	ASVs	Reads	N (%)	ASVs	Reads	N (%)
<i>Bunostomum</i>	8	61 631	12 (6%)	-	-	-
<i>trigonocephalum</i>						
<i>Chabertia ovina</i>	19	95 585	62 (32%)	-	-	-
<i>Cooperia curticei</i>	4	803	6 (3%)	-	-	-
<i>Cooperia fuelleborni</i>	2	358	5 (3%)	-	-	-
<i>Haemonchus contortus</i>	47	1 937 587	110 (56%)	38	392 110	96 (62%)
<i>Marshallagia marshalli</i>	23	338 064	57 (29%)	-	-	-
<i>Marshallagia</i> spp.	2	404	2 (1%)	-	-	-
<i>Oesophagostomum</i>	13	311 530	82 (42%)	-	-	-
<i>venulosum</i>						
<i>Ostertagia leptospicularis</i>	1	5	1 (1%)	-	-	-
<i>Ostertagia ostertagi</i>	2	269	2 (1%)	-	-	-
<i>Teladorsagia circumcincta</i>	235	3 311 094	166 (85%)	310	343 551	145 (94%)
<i>Trichostrongylus axei</i>	26	462 936	103 (53%)	33	68 414	104 (68%)
<i>Trichostrongylus</i>	12	411 776	102 (52%)	8	144 643	112 (73%)
<i>colubriformis</i>						
<i>Trichostrongylus vitrinus</i>	9	521 817	123 (63%)	44	92 834	107 (69%)
<i>Trichostrongylus</i> spp.	5	71 124	70 (36%)	-	-	-

487
 488 According to the model selection approach, host species was the only factor
 489 that explained the alpha diversity (Table S1). The model indicated that sheep had a
 490 lower alpha diversity compared to ibex ($\beta = -0.42 \pm 0.07$, $P < 0.001$, R^2 of the
 491 model=0.15) (Figure 2b). Site was not retained in the **selected model** (Table S1).

492 Beta diversity was best explained by both factors, host species ($F_{1,188} = 27.69$,
 493 $P = 0.001$) and site ($F_{3,188} = 16.39$, $P = 0.001$) and their interaction ($F_{3,188} = 25.61$, $P =$
 494 0.001) according to model selection results (Table 4, Table S2). Some gastrointestinal
 495 nematodes were mostly found in sheep feces or only in ibex feces (Figure 2a).
 496 *Trichostrongylus colubriformis* was more frequent in the Ecrins national park (mean
 497 RRA: 10% [6%; 13%]_{95CI}) than in Belledonne (mean RRA: 4% [2%; 6%]_{95CI}). Likewise,

498 *Marshallagia* spp. was more frequent in ibex feces in Cerces and Champsaur
 499 mountains (mean RRA: 11% [6%;15%]_{95CI}) than in ibex feces in Belledonne (0.4% [-
 500 0.2%; 1%]_{95CI}). The distribution of *Haemonchus contortus* in host species and sites had
 501 a particular pattern. This parasite was more frequent in ibex feces compared to sheep
 502 feces in Belledonne (mean RRA: 0.004% in sheep; 48% in ibex) and Champsaur
 503 (mean RRA: 0% in sheep; 41% in ibex), while the opposite was observed in the Cerces
 504 (Aiguillette du Lauzet: mean RRA of 7% in sheep and 0.9% in ibex; Montagne de
 505 l'Oule: mean RRA of 30% in sheep and 0% in ibex).

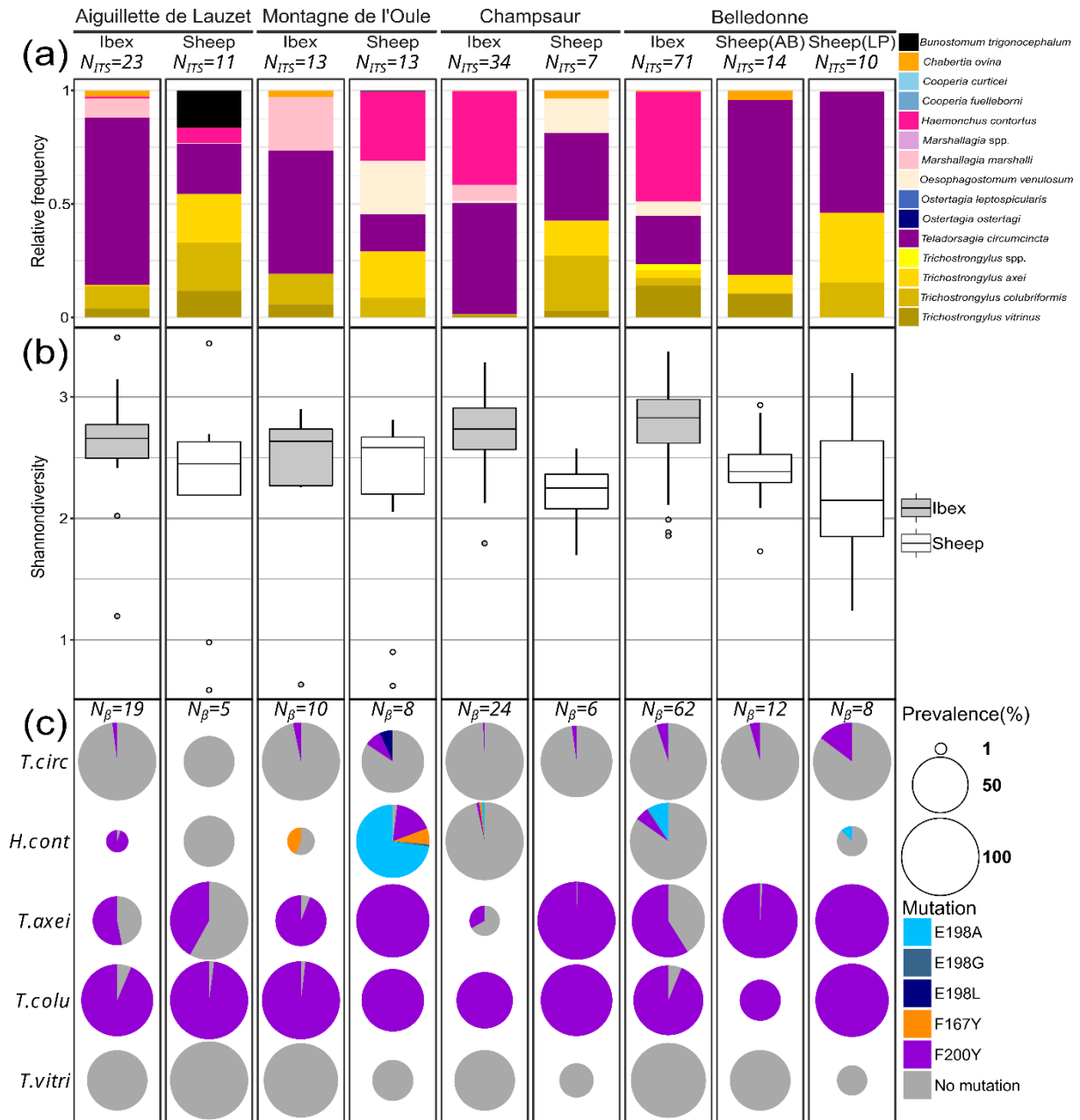
506 The model selection approach retained the effects of site and class of
 507 individuals and the interaction between the two independent factors in the best model
 508 explaining alpha diversity (Table S3). We found significant differences among sites :
 509 Shannon index of alpha diversity was higher in Belledonne ($\beta = 0.62 \pm 0.16$, $P < 0.001$,
 510 R^2 of the model=0.27) and Champsaur ($\beta = 0.55 \pm 0.16$, $P < 0.001$) compared to the
 511 Aiguillette du Lauzet. The diversity of nematodes was higher also in males compared
 512 to females/yearlings ($\beta = 0.60 \pm 0.15$, $P < 0.001$), except in Champsaur where males
 513 present a lower alpha diversity than females with kids ($\beta = -0.52 \pm 0.20$, $P < 0.001$).
 514 For the beta diversity, the best model included only the site ($F_{2,62} = 15.93$, $P = 0.001$,
 515 Table S4).

516

517 **Table 4** : Parameters estimated from the best PerMANOVA model explaining the beta
 518 diversity in ibex and sheep. The effect of host species (ibex or sheep) and site
 519 (Aiguillette de Lauzet, Montagne de l'Oule, Champsaur or Belledonne) and the
 520 interaction between the two factors are reported. Partial R^2 are reported with the
 521 corresponding F -value and p value (P).

Diversity index	Best model selected	Variables	partial R ²	F-value	P
Weighted UniFrac	$\beta \sim \text{Site} \times \text{Host species}$	Residuals	0.55	-	-
		Site	0.14	16.39	0.001
		Host species	0.08	27.69	0.001
		Site: Host species	0.22	25.61	0.001

522



523

524 **Figure 2** : (a) Mean relative frequencies of gastrointestinal nematode species, (b)

525 Shannon diversity of ITS2 ASVs and (c) prevalence and mean relative frequencies of

526 β -tubulin isotype 1. Results and sample size (N_{ITS} and N_{β}) are presented for each host

527 species (sheep or ibex) in each site (Cerces: Montagne de l'Oule and Aiguillette de

528 Lauzet; Champsaur and Belledonne: Ane Buyant (AB) and La Pesée (LP)).

529 On panel (c), the size of the pie chart corresponds to the prevalence of the corresponding

530 gastrointestinal nematode species in the population, and the size of each slice to the

531 mean proportion of each allele.

532 *Anthelmintic resistance*

533

534 We found 433 different β -tubulin isotype 1 ASVs in 154 (n= 39 sheep and n=115
535 ibex) out of 209 samples for which DNA was extracted. Among the 5 gastrointestinal
536 nematode species targeted by specific primers, we detected, *Haemonchus contortus*
537 in 96 samples, *Teladorsagia circumcincta* in 145 samples, *Trichostrongylus axei* in 104
538 samples, *Trichostrongylus vitrinus* in 107 samples and *Trichostrongylus colubriformis*
539 in 112 samples (Table 3, Figure S5). No resistance mutation was detected for
540 *Trichostrongylus vitrinus*. Therefore, *Trichostrongylus vitrinus* was not included in the
541 model explaining the relative abundance of resistant reads.

542 Resistance mutations were highly frequent (93.5%; n=144/154) with only 10
543 ibex feces (3 from Belledonne and 7 from Champsaur) in which no resistant mutation
544 was detected. Based on the best model for resistant RRA, the frequency of resistant
545 nematodes depended on gastrointestinal nematode species and the interaction
546 between host species and the study site (Table 5, Table S5). *Teladorsagia*
547 *circumcincta* was the species with the lowest resistant RRA and *Trichostrongylus*
548 *colubriformis* had the highest resistant RRA (Table 5). The mean observed RRA of
549 resistant nematodes differed between gastrointestinal nematode species
550 (*Haemonchus contortus*: 19% [13;25]_{95CI}; *Teladorsagia circumcincta*: 4% [3;6]_{95CI};
551 *Trichostrongylus axei*: 70% [63;78]_{95CI}; *Trichostrongylus colubriformis*: 96%
552 [93;99]_{95CI}). Resistant RRA were generally lower in ibex compared to sheep ($\beta = -2.21$
553 ± 0.66 , $P < 0.001$). Resistant RRA were the lowest in the Aiguillette de Lauzet but this
554 was also the only site where ibex had a significantly higher resistant RRA compared to
555 sheep (Table 5). We found no significant effect of ibex classes (males or females and
556 kids/yearlings) on benzimidazole resistance frequencies ($F_{1,49}=0.03$, $P = 0.863$,
557 ANOVA test).

558 The most frequent resistance mutation was the F200Y, present in the 4
559 gastrointestinal nematode species and 141 fecal samples, followed by the E198A (46
560 samples, 2 nematode species: *Haemonchus contortus* and *Teladorsagia*
561 *circumcincta*), the F167Y (17 samples, 2 nematode species: *Haemonchus contortus*
562 and *Teladorsagia circumcincta*), the E198L (7 samples, *Teladorsagia circumcincta*)
563 and the E198G (4 samples, *Haemonchus contortus*) (Figure 2c).

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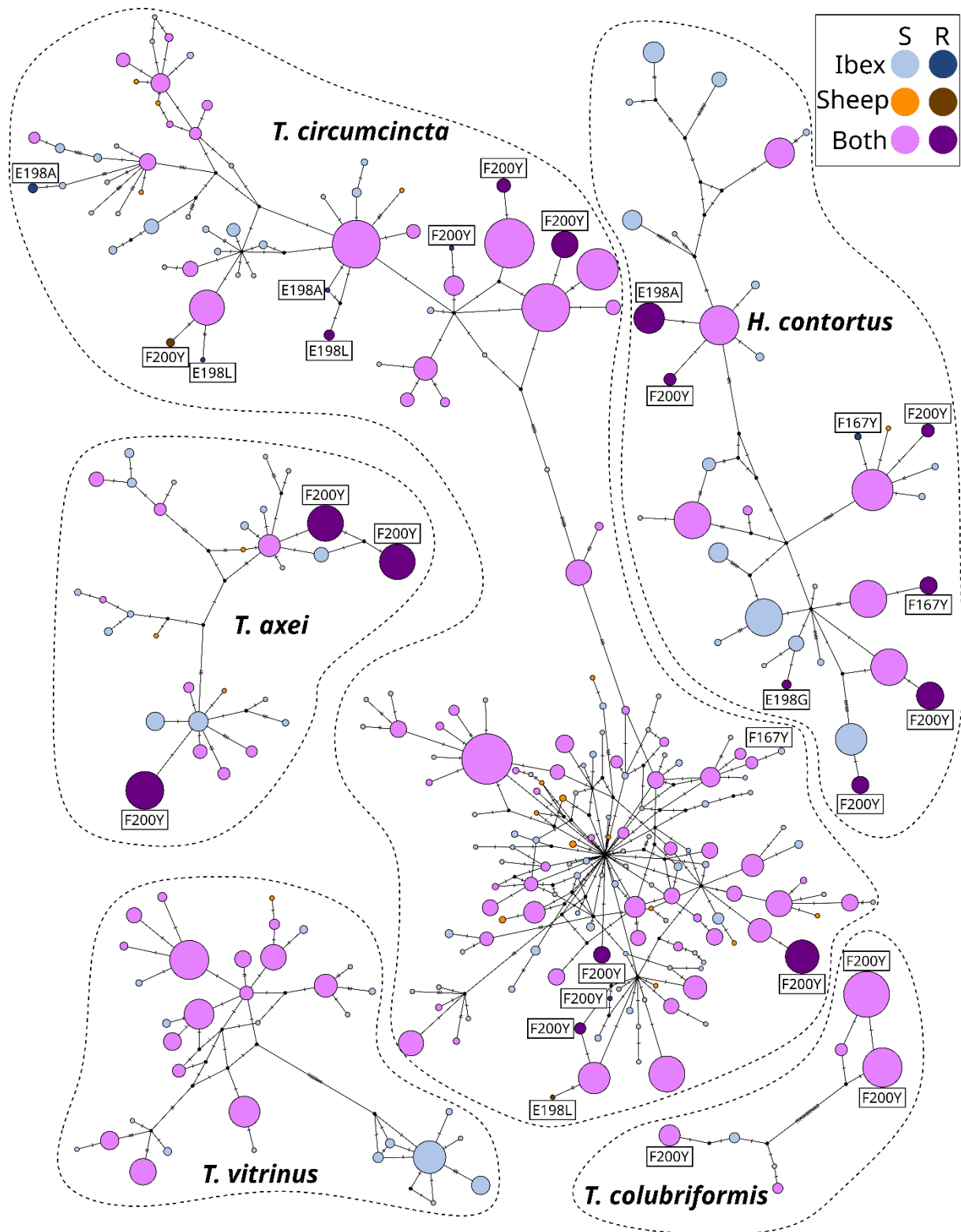
566

567 **Table 5** : Parameter estimates for the best generalized linear model explaining the
 568 resistant reads relative abundance (RRA) in ibex and sheep. The effect of host species
 569 (sheep as reference), study sites (Belledonne as reference), and their interaction, in
 570 addition to the nematode species (*Teladorsagia circumcincta* as reference) are
 571 reported. Parameter estimates with standard error (SE) are reported with the
 572 corresponding *z-value* (*z-val*) and *p value* (*P*). AL: Aiguillette de Lauzet; MO: Montagne
 573 de l'Oule; Ch: Champsaur; Hc : *Haemonchus contortus*; Ta: *Trichostrongylus axei*;
 574 Tcol: *Trichostrongylus colubriformis*.

Best model selected	Variables	Parameter estimate \pm SE	z-val	<i>P</i>
Resistant RRA ~ host species x study sites + nematode species	Intercept	-2.02 \pm 0.59	-3.45	5e-04
	Species	-2.21 \pm 0.66	-3.35	8e-04
	Mountain(AL)	-2.89 \pm 0.99	-2.93	0.003
	Mountain(MO)	-1.37 \pm 0.89	1.54	0.123
	Mountain(Ch)	-0.10 \pm 1.18	-0.08	0.935
	Nematode(Hc)	2.52 \pm 0.62	4.05	5e-05
	Nematode(Ta)	4.68 \pm 0.59	7.95	1e-15
	Nematode(Tcol)	7.38 \pm 0.75	9.79	<2e-16
	Species:Site(AL)	2.90 \pm 1.19	2.44	0.015
	Species:Site(MO)	0.04 \pm 1.28	0.03	0.973
	Species:Site(Ch)	-0.75 \pm 1.32	-0.56	0.574

575
 576 Sheep and ibex shared 164 (38%) β -tubulin isotype 1 haplotypes and 238 (55%)
 577 β -tubulin isotype 1 haplotypes were only found in ibex samples (Figure 3). Most of the
 578 resistant haplotypes of *Teladorsagia circumcincta* and *Haemonchus contortus*, e.g.,
 579 containing a non-synonymous mutation at the codon 167, 198 or 200, were genetic
 580 variants of a common sensitive haplotype shared by ibex and sheep (Figure 3). The
 581 resistant haplotypes of *Trichostrongylus axei* and *Trichostrongylus colubriformis* were
 582 more common than the sensitive haplotypes and the most similar sensitive haplotypes
 583 were found either in both sheep and ibex samples, or only in ibex samples. Both,
 584 *Trichostrongylus colubriformis* and *Trichostrongylus vitrinus* showed two distinct
 585 lineages, separated by ≥ 10 mutations. One of the lineages of *Trichostrongylus vitrinus*
 586 was only found in ibex from Belledonne and an ibex from Champsaur (Figure 3) while
 587 the ASVs of the second lineage were found both in ibex and sheep. One of the lineages
 588 of the *Teladorsagia circumcincta* was more diverse (Figure 3).

589



590

591 **Figure 3** : Median joining network of β -tubulin isotype 1 haplotypes. Each point
 592 represents a unique haplotype, and the colors correspond to the host species in which
 593 the haplotype was detected. The size of the point is proportional to the number of
 594 samples in which the haplotype was found. S: sensitive haplotype, R: resistant

595 haplotype. The tag above the points indicates the name of the mutation, based on the
596 codon position and the substitution of the amino acid.

597

598

Discussion

599

600 Because resident Alpine ibex use pastures grazed by transhumant sheep during
601 summer, but not concurrently (unpublished data), we sought to assess the extent of
602 nematode sharing between these two host species. Specifically, we investigated the
603 presence of anthelmintic-resistant nematode strains in sheep and ibex to determine
604 the role of transhumant sheep in contaminating alpine pastures, and whether ibex may
605 play a role in the circulation and maintenance of anthelmintic resistant nematodes. We
606 used a metabarcoding approach based the sequencing of ITS2 and β -tubulin to
607 demonstrate that both sheep and ibex were infected by the same gastrointestinal
608 nematode species and shared anthelmintic-resistant strains, despite the absence of
609 sheep on alpine pastures for much of the year and therefore a narrow temporal window
610 for contamination.

611 In line with other studies investigating the gastrointestinal nematodes of sheep and
612 ibex (Burgess et al., 2012; Gruner et al., 2006; Redman et al., 2019; Zaffaroni et al.,
613 2000), the most prevalent and abundant species in both host species was
614 *Teladorsagia circumcincta*. Next, *Trichostrongylus vitrinus* was moderately prevalent,
615 but not abundant in sheep and ibex nemabiomes. In accordance with the climatic
616 conditions of year-round ibex environment, these two nematode species, as well as
617 *Marshallagia* spp., are better adapted to cold temperatures than the other nematode
618 species detected in this study (O'Connor et al., 2006; Zaffaroni et al., 2000). The
619 studied sheep flocks originate from the French plains and/or the south of France and
620 are driven into mountain areas in early summer. Consequently, their nemabiome at the
621 time of sampling is representative of the gastrointestinal nematode communities
622 present in sheep on the farm, i.e., prior to transhumance. In a similar context, Gruner
623 et al., (2006) observed a high prevalence of *Teladorsagia circumcincta* in two of three
624 transhumant sheep flocks at the beginning of the grazing season in the southern Alps.
625 Furthermore, transhumant sheep flocks appear to ingest mainly *Teladorsagia*
626 *circumcincta* when grazing in the mountains, as this parasite remains the dominant
627 species identified in feces and tracer lambs during the summer (Gruner et al., 2006).
628 Pastoral activity in mountainous areas of France could therefore favor nematode

629 species more adapted to cool and wet environmental conditions, such as *Teladorsagia*
630 *circumcincta* (O'Connor et al., 2006), compared with sheep grazing on the plains year
631 round. To confirm this hypothesis, the nemabiome of transhumant sheep should be
632 compared with the nemabiome of resident sheep that stay in farm all the year around.

633 High relative frequency (>30%) of *Haemonchus contortus* was detected in ibex in
634 Belledonne and Champsaur. In contrast, almost no *Haemonchus contortus* was
635 observed in sheep flocks driven to these mountains, raising the possibility that ibex
636 may be contributing to the infection of sheep with *Haemonchus contortus*. To our
637 knowledge, this is the first time that such relative abundance of *Haemonchus contortus*
638 is reported in Alpine ibex (see previous studies based on morphological identification:
639 Carcereri et al., 2021; Marreros et al., 2012; Zaffaroni et al., 2000). In addition, it should
640 be noted that Alpine ibex were sampled before a potential contamination by domestic
641 sheep could be detected, i.e., before the end of the pre-patent period (time between
642 infection and the eggs production) and at the end of spring – early summer, i.e., the
643 start of the epidemiological period for *Haemonchus contortus* infection in high-altitude
644 mountain areas. We can therefore expect higher levels of contamination in late
645 summer, when domestic sheep leave mountain pastures. In addition, we cannot
646 exclude that some laboratory issues might have reduced the apparent prevalence and
647 abundance of *Haemonchus contortus* as some samples from sheep were kept in the
648 fridge at 4°C during 2 to 3 days (including e.g., the sheep samples without
649 *Haemonchus contortus* from Belledonne and Champsaur) which could have reduced
650 the proportion of *Haemonchus contortus* eggs hatching (McKenna, 1998).

651 The detection of *Haemonchus contortus* raises conservation issues for Alpine ibex
652 as this nematode species is known to be highly pathogenic sheep (Taylor et al., 2015).
653 Infection of a phylogenetically related species, the Pyrenean ibex (*Capra pyrenaica*
654 *pyrenaica*), with a few thousand *Haemonchus contortus* resulted in severe clinical
655 signs, including extremely low weight and hemorrhagic anemia (Lavín et al., 1997). In
656 addition, *Haemonchus contortus* may have been involved, along with pneumonia, in
657 the collapse of the Northern Chamois, *Rupicapra rupicapra*, in the province of Lecco,
658 Italy from November 2000 to March 2001 (Citterio et al., 2006). As gastrointestinal
659 nematodes can have an impact on the demographic dynamics of the host population
660 (Acerini et al., 2022; Alberly et al., 2021; Albon et al., 2002), they are suspected of being
661 behind the low natality rates observed in the French Alpine ibex populations (Brambilla
662 et al., 2020). While Alpine ibex appears to be fairly resilient to parasite infections

663 (Marreros et al., 2012), further investigations should be carried out to assess the
664 consequences of gastrointestinal nematode infections for ibex at both individual and
665 population levels.

666 Several nematode species are common to several ungulate species present in the
667 study areas (Mediterranean mouflon, *Ovis gmelini musimon* × *Ovis sp.*; Northern
668 chamois; domestic goat, *Capra hircus*; red deer, *Cervus elaphus*; and roe deer,
669 *Capreolus capreolus*) (Zaffaroni et al., 2000). In our study, only a few domestic goats
670 are present in Belledonne (n = 11 individuals) and in Champsaur (n =5 individuals) and
671 represent less than 0.01% of the domestic flock in the area. As we collected feces
672 directly on the ground, we cannot exclude that goat feces had been collected instead
673 of sheep feces. In our opinion, domestic goats should not have a significant influence
674 on nemabiome of ibex in our study area considering the scarcity of the species among
675 the sheep. In further analyses, we should consider the different domestic and wild
676 ungulates species leaving in the same study area. Especially because they have
677 different space use, different nemabiome and should provide key information to better
678 understand the dynamic of nematodes exchanges among domestic and wild
679 ungulates.

680 Contrary to results obtained on roe deer *Capreolus capreolus* (Beaumelle et al.,
681 2021), we found higher diversity of nematodes in adult males compared to females
682 and kids/yearlings. In fact, ibex have high sexual dimorphisms and male are certainly
683 more susceptible to parasitism (Markle and Fish, 2014). In addition, ibex segregate by
684 sex (Brambilla et al., 2022), providing less opportunities for intersexual transmission of
685 parasites. Contrary to females and kids, before the grazing period males feed on
686 patches grazed by domestic sheep (Margaillan, 2021), increasing the probability of
687 infection of males by over-wintering nematodes deposited by livestock during the
688 previous transhumance (O'Connor et al., 2006). On another side, we did not notice
689 any difference between classes concerning benzimidazole resistance frequencies.
690 However, more information regarding the spatial distribution of both sexes are required
691 if we want to investigate further the susceptibility of one group (male or female with
692 kids) to spread and exchange parasites with domestic livestock (Bourgoin et al., 2021).

693 We detected anthelmintic resistant alleles in 4 out of the 5 nematode species
694 tested, namely *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus*
695 *axeii*, *Trichostrongylus colubriformis*, but not *Trichostrongylus vitrinus*. Both sheep and
696 ibex hosted resistant strains of the 4 nematode species and only 10 out of 116 ibex

697 carried only susceptible strains. The benzimidazole resistance was therefore very
698 common in both host groups, in agreement with the situation of sheep farms in Europe
699 (Rose et al., 2015; Rose Vineer et al., 2020). The presence of anthelmintic resistant
700 nematodes in ibex is most likely explained by the indirect transmission of resistant
701 nematodes from sheep to ibex through the environment. The large number of shared
702 β -tubulin ASVs between sheep and ibex and the high overlap between their
703 nemabiomes confirm this scenario (Figure 2c, Figure 3). This is in accordance with
704 other studies investigating shared nematode parasites at the interface of wild and
705 domestic ungulates (Beaumelle et al., 2022; Cerutti et al., 2010; Laca Megyesi et al.,
706 2019). Whereas sheep are generally treated just before their ascent to the mountain
707 pastures, excretion of anthelmintic via sheep feces can occur during several days after
708 the drug administration and the molecules degradation last days, or even months
709 (Kolar et al., 2006). In addition, sub-lethal exposition of nematode to anthelmintic
710 residues present in the environment may select in situ for anthelmintic resistance
711 (Dimunová et al., 2022). Unfortunately, the level of drugs in the environment, their
712 persistence and their spread in grazed mountainous area are totally unknown.
713 Environmental circulation of anthelmintic residues should be investigated in further
714 studies to understand its incidence on the presence of resistant nematodes in wildlife.

715 It is worth noting that feces of ibex were sampled before the arrival of sheep on
716 pastures. This demonstrates that anthelmintic resistant nematodes can be maintained
717 in mountainous areas from year to year in wild populations of ibex despite harsh winter
718 environmental conditions, and in the absence of the main source of parasites during
719 most of the year, i.e., the domestic sheep. The shedding of eggs from resistant
720 nematodes by ibex prior to the arrival of domestic sheep suggests the potential role of
721 ibex as a reservoir of anthelmintic resistant nematodes for other susceptible domestic
722 and wild ungulates. Once resistant strains have been selected, the absence of
723 selection pressure (i.e. absence of the use of anthelmintics) do not guarantee the
724 reversion of resistance (Hamilton et al., 2022; Leathwick et al., 2015). Consequently,
725 ibex could probably maintain benzimidazole-resistant strains for several years even in
726 the absence of selection pressure. In addition, the position of resistant mutant strains
727 detected in ibex at the periphery of haplotype networks (Figure 3) supports relatively
728 recent selection of benzimidazole resistance and the lack of benzimidazole resistant
729 reversions since the resistant strains were transmitted to ibex.

730 The 5 nematode species studied seemed to have different selection dynamics
731 which may reflect life history traits (Redman et al., 2015). In fact, we detected no
732 resistant allele in *Trichostrongylus vitrinus* and conversely, the proportion of
733 benzimidazole resistant strains of *Trichostrongylus axei* and *Trichostrongylus*
734 *colubriformis* were high in sheep and somewhat lower in ibex (Figure 2c). The
735 proportions of resistant *Teladorsagia circumcincta* and *Haemonchus contortus* were
736 lower compared to *Trichostrongylus axei* and *Trichostrongylus colubriformis*, excepted
737 for the *Haemonchus contortus* of the Montagne de l'Oule sheep flock. In this study
738 area, the proportion of resistant strains of *Haemonchus contortus* was very high.

739 Consistent with our study, benzimidazole-resistant strains of *Trichostrongylus*
740 *vitrinus* were rare in other studies of sheep farms (in the UK, Avramenko et al., 2019,
741 and in Canada, Queiroz et al., 2020). In contrast, high frequencies of benzimidazole
742 resistance in *Trichostrongylus axei* and *Trichostrongylus colubriformis* (between 40%
743 and 100%) were already reported in sheep; in UK, *Trichostrongylus axei*: 26-27% and
744 *Trichostrongylus colubriformis*: 53-62% (Avramenko et al., 2019); in Austria,
745 *Trichostrongylus colubriformis*: 77%-100%, (Hinney et al., 2020); in France,
746 *Trichostrongylus axei*: 63%, (Palcy et al., 2010). In contrast, Hinney et al. (2020)
747 observed that transhumant sheep flocks in the Austria Alps had a higher mean
748 frequency of the F200Y resistance allele (*Teladorsagia circumcincta*: $32.4 \pm 6.8\%$
749 (mean \pm standard error of the mean) and *Haemonchus contortus*: $91.9 \pm 3.7\%$)
750 compared to the sheep flocks of this study (*Teladorsagia circumcincta*, $6.6 \pm 3.5\%$ and
751 *Haemonchus contortus*, $69.9 \pm 14.4\%$).

752 Several factors are suspected to contribute to interspecific differences in the
753 selection of resistance strains between nematode species, including specific
754 reproductive rates, seasonal dynamics, climatic conditions in the location of sheep
755 farms, anthelmintic strategies, e.g., treatment molecules, timing and rate of
756 anthelmintic treatments, grazing management and the cost of benzimidazole
757 resistance (Hodgkinson et al., 2019; Redman et al., 2015). However, the links between
758 parasite traits and interspecific variation of resistance acquisition by gastrointestinal
759 nematodes has not been tested yet (Morgan et al., 2019). Our results suggest a few
760 clues in relation to the ecology of the nematode species.

761 Firstly, nematode species have different abilities to practice hypobiosis, i.e., the
762 ability to halt embryonic development under environmental constraints (Gibbs, 1986).
763 *Haemonchus contortus* and *Teladorsagia circumcincta* are known to arrest

764 development more frequently than *Trichostrongylus* spp. (Langrová et al., 2008), and
765 hypobiotic larvae have been shown to be less sensitive to drugs (Sargison et al., 2007).
766 Secondly, among the *Trichostrongylus* spp., *Trichostrongylus vitrinus* may have a
767 higher proportion of overwintering larvae in pastures as this species is more resistant
768 to cold temperature compared to *Trichostrongylus axei* and *Trichostrongylus*
769 *colubriformis* (O'Connor et al., 2006). As parasites on pastures are not subject to
770 selection pressure by anthelmintics, they are a source of susceptible strains.

771 As the proportion of resistant strains is generally lower in ibex compared to sheep,
772 ibex may have contributed to a dilution effect of resistant strains, i.e., by hosting
773 susceptible nematodes. However, the role of ibex in the maintenance of a refugia
774 needs to be investigated by considering the relative number of susceptible strains
775 deposited by ibex on a pasture compared with sheep. Furthermore, it seems that the
776 role of ibex in the maintenance of a refugia may vary according to nematode species.
777 For example, ibex excrete a lower proportion of *Trichostrongylus axei* eggs than sheep
778 (Figure 2a), but containing largely resistant strains (Figure 2c). In contrast,
779 *Teladorsagia circumcincta* and *Haemonchus contortus* in ibex were more frequently
780 susceptible and genetically diverse (higher number of ITS2 and β -tubulin ASVs)
781 compared with *Trichostrongylus axei* and *Trichostrongylus colubriformis* ibex (Table 3,
782 Figure 3). As *Teladorsagia circumcincta* was dominant in ibex, a refuge of susceptible
783 *Teladorsagia circumcincta* strains may be maintained within ibex and may contribute
784 to limiting the spread of resistance in sheep farms. Nematodes can negatively or
785 positively interact within the host gut, and interactions between species or between
786 strains may have important implication for the selection of resistance. However, the
787 magnitude of within-host interactions between nematode strains/species and their
788 implication in the management of resistance remains to be determined (Hellard et al.,
789 2015; Lello et al., 2004).

790 Differences in patterns among massifs were observed at the community and
791 genetic level among sheep flocks and ibex populations. Indeed, it was expected that
792 some differences in nemabiome composition would be observed between the massifs
793 and sheep flocks, given that sheep and ibex from different areas never meet (R. Papet,
794 C. Toïgo, E. Vannard, Pers. communication). Furthermore, the sheep flocks come from
795 different locations and have been subjected to different anthelmintic strategies. For the
796 ibex, differences in the original population of translocated animals (Gauthier and
797 Villaret, 1990; Kessler et al., 2022) and potential founder effects – not all parasites

798 present in the source population were present in the newly established population -
799 may have had a long term impact on the composition of the nemabiome. For example,
800 the distinct population of *Trichostrongylus vitrinus*, found mainly in ibex in Belledonne,
801 may have been inherited from the founding ibex population. This highlights that various
802 reintroductions of ibex in the study area can also influence the composition of the
803 parasite community. This distinct population of *Trichostrongylus vitrinus* was absent in
804 sheep grazing in Belledonne, which hosted other strains of *Trichostrongylus vitrinus*
805 despite summers of co-occurrence of sheep and ibex in Belledonne. It is possible that
806 this strain is adapted to ibex and incapable of developing in sheep or that this strain is
807 highly sensitive to antiparasitic treatments used in sheep farm and systematically
808 eliminated when sheep are treated. but this observation may also be due to a sampling
809 bias as the number of sheep sampled remains low. Within the same mountain area,
810 few differences were observed among the nemabiomes of ibex, e.g., between the ibex
811 of the Aiguillette de Lauzet and those of the Montagne de l'Oule, whereas the sheep
812 flocks hosted distinct nemabiome communities.

813 In conclusion, transmission of gastrointestinal nematode species, including
814 resistant nematode strains, occur between sheep and ibex even though the contact
815 between the two species is limited to the summer period. In this study, we
816 demonstrated more specifically that ibex can maintain and shed eggs of resistant
817 gastrointestinal nematodes despite the absence of sheep on pastures for several
818 months, suggesting a potential role of ibex as a reservoir for these nematodes.
819 However, the extent to which each host species can influence the nematode
820 community of the other during the transhumant period remains to be determined. To
821 this end, a temporal sampling, before, during and after the different host species share
822 the same pasture should be considered. Analysis of parasite population structure using
823 appropriate genetic markers (i.e., microsatellites or SNPs) should help to properly
824 quantify gene flow between ibex and sheep nematode populations (Cerutti et al.,
825 2010). In addition, intervention studies are required to infer the role of ibex in
826 maintaining nematodes populations shared between the two host species (Viana et al.,
827 2014). Experimental infections of captive ibex or monitoring free-ranging ibex
828 populations after access to alpine pastures has been restricted to livestock should help
829 us to refine the ability of ibex to maintain nematodes from domestic ungulates,
830 including resistant nematodes. Finally, epidemiological models could be useful tools to
831 better understand the dynamics of resistant parasites at the livestock-wildlife interface

832 (Brown et al., 2022; Dickinson et al., 2024). The lower proportion of resistance alleles
833 in ibex compared to sheep underlines the possibility that ibex could contribute to the
834 maintenance and circulation of susceptible strains in sheep. Based on our results, it
835 seems that ibex have helped to limit the spread of anthelmintic resistance in
836 *Teladorsagia circumcincta* and *Haemonchus contortus* in sheep flocks, by maintaining
837 parasite refugia not exposed to anthelmintic pressure. As with roe deer (Beaumelle et
838 al., 2022), domestic sheep contribute to the modification of the nemabiome of ibex.
839 This raises concerns about ibex conservation, and the consequences of strongyles
840 infection in ibex should be investigated. Indeed, ibex are characterized by low genetic
841 diversity due to the strong demographic decline of this species followed by multiple re-
842 introductions (Grossen et al., 2018). The high genetic structure of immunity-related loci
843 among ibex populations (Kessler et al., 2022) raised additional concerns, whereas both
844 neutral and adaptive genetic diversity are known to have an influence on parasites
845 resistance in ungulates (Portanier et al., 2019).

846

847

Appendices

848

849 Supplementary data to this article can be found online in MendeleyData (DOI:
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868

869 **Conflict of interest disclosure**

870

871 The authors declare that they have no conflict of interest.

872

873 **Data, scripts, and supplementary information availability**

874

875 The bioinformatic pipeline, the ASV analysed during the current study and the R script
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877

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