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# Cross-transmission of resistant gastrointestinal nematodes between wildlife and transhumant sheep

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34 **ABSTRACT**

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

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

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

61

62 **Keywords:** ITS-2 rDNA, benzimidazole resistance; isotype-1  $\beta$ -tubulin; livestock;  
63 nemabiome metabarcoding; wild ungulates, transhumant sheep, Alpine ibex

64

65

66

## Introduction

67

68 Parasites represent a large proportion of animal diversity and are key components  
69 of food webs (Hudson et al., 2006) and are essential determinants of the health,  
70 fitness, population dynamics and community composition of their hosts (Tompkins et  
71 al., 2011). The parasites of the Nematodes class infect a wide range of species  
72 worldwide, including animals and plants (Blaxter and Koutsovoulos, 2015). In  
73 animals, the gastrointestinal nematode parasites are of major concern for livestock  
74 productivity and security as they can impact animal health implying reduced animal  
75 productions and economical losses (Charlier et al., 2020; Roeber et al., 2013).

76 To limit the parasite load and its impact on livestock health, the use of  
77 anthelmintics to treat livestock against gastrointestinal nematodes is a common and  
78 cost effective practice (Vercruysse et al., 2018). Nonetheless, the repeated use of  
79 anthelmintics has led to the selection of anthelmintic-resistant strains of  
80 gastrointestinal nematodes. Within the gastrointestinal nematodes, resistances to  
81 several families of anthelmintics (e.g., benzimidazole, macrocyclic lactones and  
82 levamisole) has been observed, and resistance to several drugs is increasing  
83 (Bordes et al., 2020; Kaplan and Vidyashankar, 2012; Rose et al., 2015; Rose Vineer  
84 et al., 2020). The first case of resistance to benzimidazole was reported in 1964 in  
85 lambs from central Kentucky (Drudge et al., 1964).

86 Today, resistance to benzimidazoles is widespread throughout the world (Kaplan  
87 and Vidyashankar, 2012), and is particularly common on sheep farms in Europe  
88 (Papadopoulos et al., 2012; Rose Vineer et al., 2020). The mechanisms of resistance  
89 to benzimidazole are well known and documented (Whittaker et al., 2017). The  
90 anthelmintic effect of benzimidazoles relies on the fixation of the molecule to the  
91 parasite  $\beta$ -tubulin isotype-1, resulting in the disruption of tubulin–microtubule  
92 equilibrium (Whittaker et al., 2017). In resistant nematodes, specific mutations of the  
93  $\beta$ -tubulin isotype-1 gene have been correlated with the resistance to benzimidazole in  
94 several gastrointestinal nematode species (Charlier et al., 2022). These mutations  
95 were associated with alteration of the  $\beta$ -tubulin isotype-1 structure, decreasing the  
96 affinity of the protein with the benzimidazole and subsequently, activity of  
97 benzimidazole (Whittaker et al., 2017).

98 Some generalist gastrointestinal nematodes can infect several host species  
99 (Walker and Morgan, 2014), including both domestic and wild ungulates (e.g.,

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

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

126 Spatial segregation between wild and domestic ungulates is usually observed  
127 once livestock arrive on pasture (Brivio et al., 2022; Ryser-Degiorgis et al., 2002).  
128 Livestock are generally released onto the best grazing areas during the summer  
129 season due to their high productivity and accessibility of water for animals, and salt  
130 licks provides essential mineral nutrients in relatively nutrient-poor alpine ecosystems  
131 (Chirichella et al., 2014; Richomme et al., 2006; Ryser-Degiorgis et al., 2002).  
132 However, prior to the arrival of livestock, mountain ungulates have been observed  
133 using preferentially the same grazing areas as those used by livestock on their arrival

134 (Brivio et al., 2022; Ryser-Degiorgis et al., 2002) and ibex, for example, have been  
135 observed to return to the sheep grazing area immediately after the sheep have left  
136 (Ryser-Degiorgis et al., 2002). The presence of wild and domestic ungulates in  
137 attracting zones such as salt licks, even if not simultaneous, offers good opportunities  
138 for parasites transmission, and these areas are therefore considered hotspots for  
139 parasites infection (Richomme et al., 2006; Ryser-Degiorgis et al., 2002; Utaaker et  
140 al., 2023).

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

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

162 Given that the co-occurrence of sheep and ibex on the same pastures is limited to  
163 the summer period and since sheep, and that ibex usually host species-specific  
164 gastrointestinal nematodes (Walker and Morgan, 2014), we expect the nemabiome to  
165 be highly differentiated between the two species in the three mountain areas (H1).  
166 We expect sheep to host benzimidazole-resistant strains of gastrointestinal  
167 nematode, in line with the general pattern observed for sheep in France

168 (Papadopoulos et al., 2012; Rose Vineer et al., 2020). With the implementation of  
169 reintroduction programs in the second half of the 20<sup>th</sup> century, ibex have colonized  
170 pastures traditionally grazed by sheep. We therefore expect that ibex will also host  
171 benzimidazole-resistant gastrointestinal nematodes but to a lesser extent, as  
172 resistance do not represent a selective advantage for nematodes in the ibex  
173 environment (Hahnel et al., 2018) (H2). Because there are very few documented ibex  
174 dispersal events among the 3 ibex populations ((Brambilla, 2020), R. Papet, C. Toïgo  
175 and E. Vannard, personal communication), we should observe genetic differences  
176 among nematodes species/community or strains (ASV) among the populations of  
177 ibex due to genetic drift (H3).

178

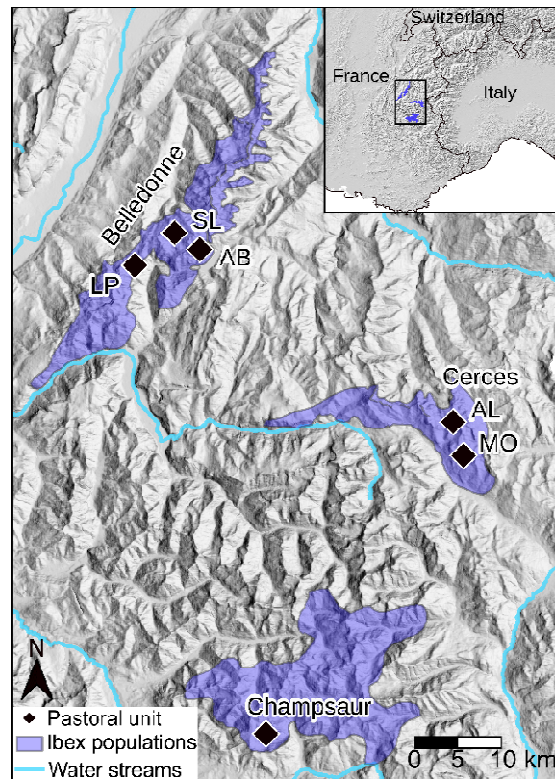
## 179 **Materials and Methods**

180

### 181 *Study area*

182

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



188

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

193

194 The 3 study areas are characterized by the presence of steep slopes, high  
195 peaks (>2500m) and agropastoral activities. Climatic conditions are harsh in these  
196 mountains with a mean temperature in winter (December-March) 2015-2019 of 0°C  
197 in Belledonne (alt:1785m), 1°C in Champsaur (alt:1620m) and 1.5°C in Cerces  
198 (alt:1553m). During summer (June-September), the mean temperature is 13°C in  
199 Belledonne, 14°C in Champsaur and 16°C in the Cerces (réseau d'observation  
200 météo du massif alpin). Champsaur is the southern study area and has a  
201 Mediterranean influence. Consequently, rainfall is less important in this area  
202 compared to Cerces and Belledonne. The vegetation is distributed along an elevation  
203 gradient from coniferous woodland (*Abies alba* and *Picea abies* in Belledonne and  
204 *Larix decidua* and *Pinus sylvestris* in Cerces and Champsaur) in the lower range of  
205 ibex, to a landscape dominated by heathland with *Rhododendron ferrugineum*,



206 *Vaccinium* spp. and *Juniperus communis*, and grassland (*Carex* spp. *Festuca* spp.)  
207 above the tree line (Ozenda, 1985).

208 The ibex populations were established in Belledonne, Cerces and Champsaur,  
209 in 1983 with the introduction of 20 ibex, in 1959-1961 with the introduction of 6 ibex  
210 and in 1994-1995 with the introduction of 30 ibex, respectively.

211 The distribution of ibex in Belledonne, Cerces and Champsaur range between  
212 630m and 2860m on 200km<sup>2</sup>, between 1410m and 3100m on 120km<sup>2</sup> and between  
213 1320m and 3550m on 280km<sup>2</sup>, respectively. Census populations sizes are estimated  
214 to 800, 320 and 420 individuals in Belledonne, Cerces and Champsaur, respectively.

215 Traditional pastoral activity is practiced in all massifs, where sheep flocks  
216 arrive early summer to graze mountain pastures coming from the plain on foot or by  
217 truck. In Belledonne, the size of the herds are 750 ewes followed by their lambs in La  
218 Pesée, 900 ewes in Sept Laux, and 1600 ewes in Ane Buyant. A dozen of rams are  
219 also present within the La Pesée and Sept Laux herds, as well as some goats in La  
220 Pesée. Each sheep herd from Belledonne belongs to one farmer while several  
221 farmers grouped their sheep herds in Cerces and Champsaur. In Champsaur, the  
222 herd included 4 breeding farms for a total of 1070 sheep and 5 goats. In Cerces, the  
223 herd located in the West (Aiguillette du Lauzet) included 3 breeding farms for a total  
224 of 800 sheep and the herd located in the East (Montagne de l'Oule) included 4  
225 breeding farms for a total of 940 sheep.

226

## 227 *Sample collection*

228

229 We collected sheep feces up to 15 days after arrival on pasture to ensure that  
230 we collected nematode species representative of sheep at the time of arrival on  
231 pastures and to avoid detecting nematodes ingested secondarily on alpine pastures.  
232 Similarly, ibex feces were collected prior to the arrival sheep and until 15 days after  
233 arrival to ensure that the nematode community was not yet influenced by the arrival  
234 of domestic livestock. Fresh ibex feces were mostly collected directly on the ground  
235 and, in Belledonne, also during captures as part of the long-term monitoring program  
236 conducted by the French Office for Biodiversity. Where possible, feces were collected  
237 immediately after observation of ibex to avoid collecting of feces from the same  
238 individual. Samples were stored in plastic bags, sealed after air removal, and

239 analyzed within 48h upon receipt maximum 15 days after field work (mean: 2.5 [min:  
240 0 - max: 15] days) in the parasitology laboratory of the National Veterinary School of  
241 Lyon (ENVL, Marcy-l'Étoile, France). In total, we sampled 167 fecal samples from  
242 ibex and 90 fecal samples from 6 sheep herds, distributed over four study sites, i.e.,  
243 Belledonne, Champsaur, Aiguillette du Lauzet, Cerces and Montagne de l'Oule,  
244 Cerces (Table 1, Figure S1).

245

246 **Table 1** : Location, period and number of samples collected in the French alps.

247 Coproscopic results of the gastro-intestinal (GI) nematodes (strongyles) eggs are  
248 also reported (med[min-max]). Cerces : AL: Aiguillette de Lauzet, and MO: Montagne  
249 de l'Oule ; Belledonne : AB: Ane Buyant, LP : La Pesée and SL : Sept Laux.

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

250

## 251 *Parasitological analyses*

252

253 The number of gastro-intestinal nematodes eggs per gram of feces (epg) was  
254 counted following a modified McMaster protocol (Raynaud et al., 1970). In order that  
255 strongyles reach the L3 stage, coprocultures of feces were done at  $24 \pm 1$  °C during  
256 12-15 days with regular mixing and moistening. After the collection of L3 in tape  
257 water with a Baermann apparatus, we evaluated the success of the coproculture by  
258 counting the number of L3 in each sample. We extracted gastrointestinal nematodes  
259 DNA from samples for which there were at least 20 L3 and we limited the extraction  
260 to ~200 L3. DNA was extracted using extraction kit (Qiagen DNeasy® PowerSoil)  
261 following the manufacturer's instruction with an elution volume of 50 µl of water. We

262 extracted twice the DNA of 30 randomly chosen samples as internal extraction  
263 controls. We quantified DNA concentration for all samples using Qubit 2.0  
264 fluorometer (Life Technologies) and we homogenized DNA samples to a DNA  
265 concentration of 1ng/μl (DNA samples were not diluted if the DNA concentration was  
266 <1 ng/μl).

267

### 268 *High throughput sequencing analyses*

269

270 To determine the nemabiome of sheep and ibex we used a modified version of  
271 the protocol developed by Avramenko et al., (2015). The ITS2 region of the nuclear  
272 rDNA was amplified using the primer pair NC1 (Forward - 5'-  
273 ACGTCTGGTTCAGGGTTGTT-3') and NC2 (Reverse- 5'-  
274 TTAGTTTCTTTTCCTCCGCT-3') with the following PCR conditions: 10μl of Applied  
275 Biosystems™ Master Mix AmpliTaq Gold™ 360, 5,84μl of molecular biology grade  
276 water, 0,16μl of Bovine Serum Albumin, 2μL of 5 μM mixed F and R primers form, 2  
277 μL of DNA lysate. The PCR was performed under the following conditions: 10 min  
278 initial denaturation at 95°C; 35 cycles of denaturation (30 s at 95°C), annealing (30 s  
279 at 54°C), and extension (1 min at 72°C); a final extension at 72°C for 7 min. The  
280 thermocycling parameters were choose identically to Avramenko et al., (2015). To  
281 detect the mutations responsible of the resistance of gastrointestinal nematode to  
282 benzimidazole, we used a modified protocol of Avramenko et al., (2019). We  
283 amplified the isotype-1 β-tubulin fragment comprising the codon at position 167, 198  
284 and 200 with two pairs of primers in two independent PCR.

285 The PCR conditions were: 10μl of Applied Biosystems™ Master Mix  
286 AmpliTaq Gold™ 360, 5.84 μl of molecular biology grade water, 0.16 μl of Bovine  
287 Serum Albumin, 2 μL of 5 μM mixed forward and reverse primers mix, 2 μL of DNA  
288 lysate. The PCR was performed under the following conditions: 10 min initial  
289 denaturation at 95°C; 40 cycles of denaturation (30 s at 95°C), annealing (30 s at  
290 65°C), and extension (30 s at 72°C); a final extension at 72°C for 7 min. We targeted  
291 *Teladorsagia circumcincta* and *Trichostrongylus* spp. (Forward:5'-  
292 CGCATTWCTTGGAGGAGG-3' and Reverse: 5'-  
293 GTGAGYTTCAAWGTGCGGAAG-3') and *Haemonchus contortus* (Forward:5'-  
294 CGCATTCTTGGGAGGAGG-3' and Reverse: 5'-GTGAGTTTYAAGGTGCGGAAG-

295 3') with the primers described by Avramenko et al., (2019). All forward and reverse  
296 primers were tagged at 5' in order that each sample had a unique combination of  
297 tagged primers.

298 In all PCRs, we added positive PCR controls (i.e., *Haemonchus contortus* and  
299 *Teladorsagia circumcincta* DNA extracts), negative PCR controls (distilled H<sub>2</sub>O) and  
300 negative DNA extraction controls. All samples (including controls) were  
301 independently amplified 4 times to ensure reliability of the sequencing, in 96-well  
302 plates including 209 samples, 17 PCR positive controls, 13 PCR negative controls, 7  
303 extraction negative controls, 30 DNA extraction controls, as well as 12 empty wells in  
304 each 96-well plates to quantify tag jumping during PCR and sequencing steps (Figure  
305 S2) (De Barba et al., 2014; Taberlet et al., 2018).

306 All PCR products of the ITS2 and the two  $\beta$ -tubulin isotype 1 sets were purified  
307 using QIAquick® Spin Columns (QIAquick® PCR Purification Kit QIAGEN) and  
308 quantified using a Qubit 2.0 fluorometer (Life Technologies). Next, we pooled the 3  
309 purified DNA pools (ITS2, two  $\beta$ -tubulin isotype 1) based on their initial concentration  
310 and in proportion according to the following ratio: ITS2 50%,  $\beta$ -tubulin isotype 1 25%  
311 for each. According to preliminary tests, we expect to achieve a sequencing depth of  
312 20 000 reads per ITS2 DNA sample and 5 000 reads per  $\beta$ -tubulin isotype 1 DNA  
313 sample. Sequencing was performed with pair-end sequencing technology on the  
314 Illumina platform (2\*250 bp Miseq) at Fasteris, Geneva, Switzerland.

315

### 316 *Sequence analysis and taxon assignation*

317

318 The sequence reads were first analyzed with the OBITOOLS package (Boyer et al.,  
319 2016). Forward and reverse reads were assembled with the *alignpairedend* function,  
320 and we kept only sequences with a good score of alignment (rnorm>0.8). Sequences  
321 were attributed to their samples with the *ngsfilter* function with default parameters.  
322 Subsequently, assigned sequences were analyzed with the *dada2* package  
323 (Callahan et al., 2016) following the pipeline available in [www.nemabiome.ca](http://www.nemabiome.ca). The  
324 *dada2* pipeline returns Amplicon Sequence Variant (ASV) which are sequence  
325 variants differing by as little as one nucleotide (Callahan et al., 2017). Following  
326 Beaumelle et al., (2021), the gastrointestinal nematodes were identified with three  
327 different methods of assignation: BLASTn (Altschul et al., 1990) against the NCBI

328 database (November 2022), and AssignTaxonomy (Callahan et al., 2016; Wang et  
329 al., 2007) and IDTaxa (Murali et al., 2018) against the nematode ITS2 rDNA  
330 database 1.1.0 (Workentine et al., 2020) or the nematode  $\beta$ -tubulin isotype 1 DNA  
331 reference sequence supplied in Avramenko et al., (2019). We chose to attribute a  
332 confidence level to taxonomic identification at species level: high or moderate  
333 confidence if the three or two methods of assignation, respectively, were congruent.  
334 We also adjusted the sequence filtering based on an adapted procedure of  
335 Calderón-Sanou et al., (2020). We kept only ASVs present in at least 2 replicates of  
336 the same samples and removed ASVs that were not assigned to genus level for the  
337 ITS2 and species level for  $\beta$ -tubulin isotype 1. We removed potential contaminants  
338 (reagent contaminants and cross-contaminations) following the procedure detailed in  
339 Calderón-Sanou et al., (2020). For each sample, we sum the reads of the two  
340 replicates with the highest similarity and if this similarity is higher than the mean  
341 similarity among all replicates. At the end, we removed samples if they have <1000  
342 reads of ITS2 and <500 reads of  $\beta$ -tubulin isotype 1 (Figure S3).

343

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

345

346 For each nematode species, all  $\beta$ -tubulin isotype 1 ASVs were aligned to one  
347 of the  $\beta$ -tubulin isotype 1 consensus sequences of the reference database  
348 (Avramenko et al., 2019) using the *AlignSeqs* function of the DECIPHER package  
349 (Wright, 2016). We examined each  $\beta$ -tubulin isotype 1 ASV at codon 167,198 and  
350 200 positions to record whether the codon is associated with a non-synonymous  
351 mutation. However, we ignored the other polymorphism sites of the Exon.

352

#### 353 *Statistical analyses on measures of nemabiomes*

354

355 To measure the differences of nemabiome among the two host species (sheep and  
356 ibex) and the 4 study sites (Aiguillette de Lauzet, Montagne de l'Oule, Champsaur  
357 and Belledonne), we considered two measures of diversity, i.e., the alpha diversity  
358 and the beta diversity. The alpha diversity was measured with the Shannon index  
359 that considers richness and evenness of communities and the beta diversity was  
360 measured with the weighted UniFrac index estimated using the R phyloseq package

361 (McMurdie and Holmes, 2013). The weighted UniFrac distance is a phylogenetic  
362 distance between the set of ASV from each nemabiome weighted by the transformed  
363 abundance of each ASV (Lozupone and Knight, 2005). The phylogenetic distances  
364 were computed from a phylogenetic tree which was conducted using a maximum  
365 likelihood tree with the GTR+G+I model according to the *ModelTest* function (Posada  
366 and Crandall, 1998; Schliep, 2011). The exact count of ITS2 reads were transformed  
367 with the Hellinger transformation (e.g., square root on relative frequencies) to account  
368 for the high number of zero in community table and to decrease the influence of rare  
369 ASVs in statistical analyses (Legendre and Legendre, 2012).

370 We tested the effects of host species and site, including their interaction, on alpha  
371 diversity with linear models, and on beta diversity using perMANOVA (*adonis2*,  
372 *vegan* R package (Oksanen et al., 2020)). All possible models including the null  
373 model were computed. For perMANOVA models, we used a custom function to  
374 compute Akaike's information criterion corrected for small sample size (AICc) based  
375 on residual sums of squares (Dyson, 2018). In a model selection approach, for both  
376 alpha and beta diversity, all possible models were ranked using the AICc and we  
377 selected the model with the lowest AICc value. Models with  $\Delta AICc \leq 2$  were  
378 considered equivalent (Burnham and Anderson, 2002), and in this case, we  
379 considered the most parsimonious one, i.e., the model with the lowest degree of  
380 freedom.

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

383

### 384 *Statistical analyses on measures of resistant nematode strains*

385

386 To compare the importance of benzimidazole resistance of gastrointestinal  
387 nematodes between ibex and sheep and among the 3 sites, we tested if the host  
388 species, the site and the gastrointestinal nematode species influence the relative  
389 abundance of ASVs with a resistant allele. For this purpose, we used a generalized  
390 linear model with a binomial family and a model selection approach such as  
391 described above.

392 We used *AlignSeqs* (Wright, 2016) to generate multi-sequence aligned  $\beta$ -tubulin  
393 isotype 1 haplotype data. For each gastrointestinal species, we removed short ASVs,

394 e.g., ASVs with a sequence length <10% comparing to the median ASVs length.  
395 PopART v1.7 (Leigh and Bryant, 2015) was used to draw the median joining  
396 networks based on each gastrointestinal nematode haplotypes data.

397

398

## Results

399

### *Parasite material*

400

401  
402 The median number of eggs of strongyles per gram of feces were lower in sheep  
403 (7.5[0,148]<sub>95%IQR</sub>; n = 90) than in ibex (15[0,163]<sub>95%IQR</sub>; n = 167) feces (Mann-  
404 Whitney U test;  $W = 9043.5$ ,  $P = 0.006$ ). As a result of the low level of infestation in  
405 some samples, the number of L3 hatched from eggs were not sufficient (<20) for 48  
406 ibex or sheep samples. These samples were not used for subsequent genetic  
407 investigations. Specifically, all samples from the Sept Laux sheep herd (n=15,  
408 Belledonne) were discarded. Therefore, the nemabiome was determined based on  
409 the ITS2 for 196 (n= 55 sheep and n=141 ibex) out of 209 samples for which DNA  
410 was extracted (Figure S1).

411

### *Diversity of gastrointestinal nematodes in sheep and ibex*

412

413  
414 In total, we detected 408 ASVs corresponding to 13 gastrointestinal nematode  
415 species (Table 2, Figure S4). Eight ASVs were assigned to the genus level (i.e.,  
416 *Marshallagia* spp., *Nematodirus* spp. and *Trichostrongylus* spp.) due to non-identical  
417 assignation among taxonomic methods. An ASV corresponding to the lungworm  
418 *Cystocaulus ocreatus* were discarded for the statistical analyses because we only  
419 focus on gastrointestinal nematodes.

420

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

427 The Nematodirus were not considered for the following results as our protocol is not  
 428 appropriate for this species (Hoberg et al., 2001)

429

430 **Table 2** : Number of ASV, reads and samples for each nematode taxon, including the  
 431 results from the ITS2 (nemabiome) and the  $\beta$ -tubulin isotype 1 (resistance to  
 432 benzimidazole). N= number and percentage of samples in which the taxa was  
 433 detected. Host species and study sites are mixed here.

	ITS2			$\beta$ -tubulin isotype 1		
	ASV	Reads	N (%)	ASV	Reads	N (%)
<i>Bunostomum</i> <i>trigonocephalum</i>	8	61 631	12 (6%)	-	-	-
<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> <i>venulosum</i>	13	311 530	82 (42%)	-	-	-
<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> <i>colubriformis</i>	12	411 776	102 (52%)	8	144 643	112 (73%)
<i>Trichostrongylus vitrinus</i>	9	521 817	123 (63%)	44	92 834	107 (69%)
<i>Trichostrongylus</i> spp.	5	71 124	70 (36%)	-	-	-

434

435 According to the model selection approach, the host species was the only  
 436 factor that explained the alpha diversity (Table S1). The model indicates that sheep  
 437 had a lower alpha diversity compared to ibex ( $\beta = -0.42 \pm 0.07$ ,  $P < 0.001$ ,  $R^2$  of the  
 438 model=0.15) (Figure 2b). The site was not retained in the model selected to explain  
 439 the alpha diversity (Table S1).

440 Beta diversity is best explained by both factors, host species ( $F_{1,188} = 27.69$ ,  $P$   
 441  $= 0.001$ ) and site ( $F_{3,188} = 16.39$ ,  $P = 0.001$ ) and their interaction ( $F_{3,188} = 25.61$ ,  $P =$



442 0.001) according to our model selection (Table 3, Table S2). Some gastrointestinal  
 443 nematodes were mostly (*Trichostrongylus axei*: mean reads relative abundance  
 444 (RRA) of 19% [11%; 26%]<sub>95CI</sub> in sheep feces and 2% [1%;2%]<sub>95CI</sub> in ibex feces) or  
 445 only (*Bunostomum trigonocephalum*, *Cooperia* spp.) found in in sheep feces or only  
 446 in ibex feces (*Marshallagia* spp., *Ostertagia* spp.; Figure 2a). *Trichostrongylus*  
 447 *colubriformis* was more frequent in the Ecrins national park (mean RRA: 10% [6%;  
 448 13%]<sub>95CI</sub>) than in Belledonne (mean RRA: 4% [2%; 6%]<sub>95CI</sub>). Likewise, *Marshallagia*  
 449 spp. was more frequent in ibex feces in Cerces and Champsaur mountains (mean  
 450 RRA: 11% [6%;15%]<sub>95CI</sub>) than in ibex feces in Belledonne (0.4% [-0.2%; 1%]<sub>95CI</sub>). The  
 451 distribution of *Haemonchus contortus* in host species and sites had a particular  
 452 pattern. This parasite was more frequent in ibex feces compared to sheep feces in  
 453 Belledonne (mean RRA: 0.004% in sheep; 48% in ibex) and Champsaur (mean RRA:  
 454 0% in sheep; 41% in ibex), while the opposite was observed in the Cerces (Aiguillette  
 455 du Lauzet: mean RRA of 7% in sheep and 0.9% in ibex; Montagne de l'Oule: mean  
 456 RRA of 30% in sheep and 0% in ibex).

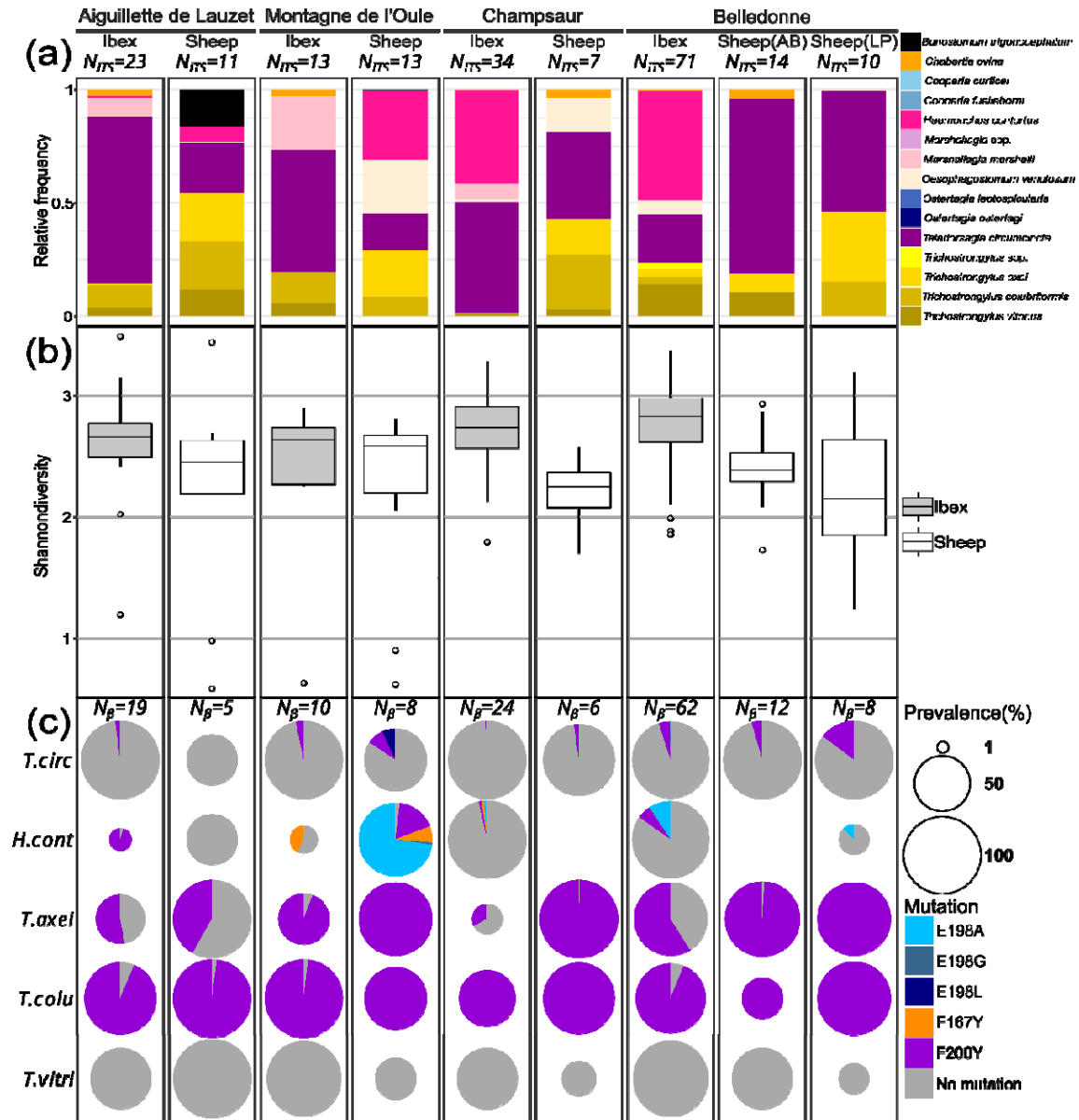
457

458 **Table 3** : Parameters estimated from the best PerMANOVA model explaining the  
 459 beta diversity in ibex and sheep. The effect of host species (ibex or sheep) and site  
 460 (Aiguillette de Lauzet, Montagne de l'Oule, Champsaur or Belledonne) and the  
 461 interaction between the two factors are reported. Partial R<sup>2</sup> are reported with the  
 462 corresponding *F*-value and *p* value (*P*).

Diversity index	Best model selected	Variables	partial R <sup>2</sup>	F-value	P
Weighted UniFrac	β ~ Site x 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

463

464



465

466 **Figure 2 :** (a) Mean relative frequencies of gastrointestinal nematodes species and  
 467 (b) Shannon diversity of ITS2 ASVs and (c) prevalence and mean relative  
 468 frequencies of  $\beta$ -tubulin isotype 1. For each host species (sheep or ibex) and each  
 469 site (Cerces: Montagne de l'Oule and Aiguillette de Lauzet; Champsaur and  
 470 Belledonne). The sample size for ITS2 ASVs ( $N_{ITS}$ ; panel (a)) and for  $\beta$ -tubulin  
 471 isotype 1 ( $N_{\beta}$ ; panels (b)) is given for each population. On the panel (c), the size of  
 472 the pie chart corresponds to the prevalence of the corresponding gastrointestinal  
 473 nematode species in the population, and the size of each sector to the mean  
 474 proportion of each allele.

475

## 476 *Anthelmintic resistance*

477

478 We found 433 different  $\beta$ -tubulin isotype 1 ASVs in 154 (n= 39 sheep and  
479 n=115 ibex) out of 209 samples for which DNA was extracted. Among the 5  
480 gastrointestinal nematode species targeted by primers, we detected, *H. contortus* in  
481 96 samples, *Teladorsagia circumcincta* in 145 samples, *Trichostrongylus axei* in 104  
482 samples, *Trichostrongylus vitrinus* in 107 samples, or *Trichostrongylus colubriformis*  
483 in 112 samples (Table 2, Figure S5). No resistance mutation has been detected for *T.*  
484 *vitrinus*. Therefore, *T. vitrinus* was not included in the model explaining the relative  
485 abundance of resistant reads.

486 Resistance mutations were highly frequent (93.5%; n=144/154) with only 10  
487 ibex feces (3 from Belledonne and 7 from Champsaur) in which no resistant mutation  
488 has been detected.

489 Based on the best model for resistant RRA, the frequency of resistant nematodes  
490 depended on gastrointestinal nematode species and the interaction between host  
491 species and the study site (Table 4, Table S3). *Teladorsagia circumcincta* was the  
492 species with the lowest resistant RRA and the nematode species with the higher  
493 resistant RRA was *Trichostrongylus colubriformis* (Table 4). The mean observed  
494 RRA of resistant nematodes differed between GIN species (*H. contortus*: 19%  
495 [13;25]<sub>95CI</sub>; *T. circumcincta*: 4% [3;6]<sub>95CI</sub>; *T. axei*: 70% [63;78]<sub>95CI</sub>; *T. colubriformis*:  
496 96% [93;99]<sub>95CI</sub>). The resistant RRA is generally lower in ibex compared to sheep ( $\beta$   
497 = -2.21  $\pm$  0.66,  $P < 0.001$ ). Resistant RRA were the lowest in the Aiguillette de Lauzet  
498 but this is also the only site where ibex had significantly higher resistant RRA than  
499 sheep (Table 4).

500 The most frequent resistance mutation is the F200Y present in the 4  
501 gastrointestinal nematode species and 141 feces samples, followed by the E198A (46  
502 samples, 2 nematode species: *H. contortus* and *T. circumcincta*), the F167Y (17  
503 samples, 2 nematode species: *H. contortus* and *T. circumcincta*), the E198L (7  
504 samples, *T. circumcincta*) and the E198G (4 samples, *H. contortus*) (Figure 2c).

505

506 **Table 4** : Parameter estimates for the best generalized linear model explaining the  
507 resistant reads relative abundance (RRA) in ibex and sheep. The effect of host  
508 species (sheep as reference), study sites (Belledonne as reference), and their  
509 interaction, in addition to the nematode species (*Teladorsagia circumcincta* as

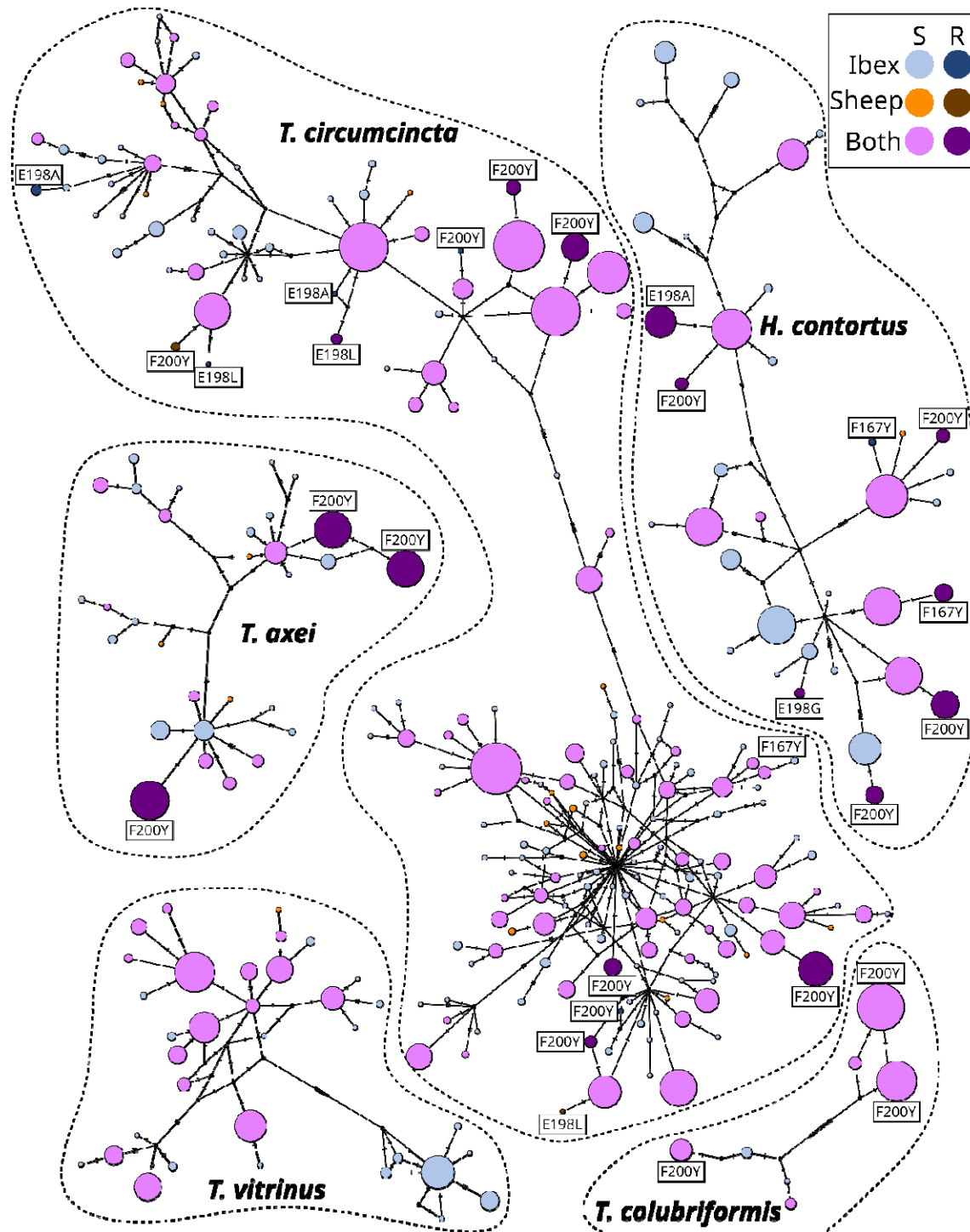
510 reference) are reported. Parameter estimates with standard error (SE) are reported  
 511 with the corresponding *z-value* (*z-val*) and *p value* (*P*). AL: Aiguillette de Lauzet; MO:  
 512 Montagne de l'Oule; Ch: Champsaur; Hc: *Haemonchus contortus*; Ta:  
 513 *Trichostrongylus axei*; Tcol: *Trichostrongylus colubriformis*.

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

514

515 Sheep and ibex shared 164 (38%)  $\beta$ -tubulin isotype 1 haplotypes and 238  
 516 (55%)  $\beta$ -tubulin isotype 1 haplotypes was only found in ibex samples (Figure 3). Most  
 517 of the resistant haplotypes of *T. circumcincta* and *H. contortus*, e.g., containing a  
 518 non-synonym mutation to the codon 167, 198 or 200, were genetic variants of a  
 519 common sensitive haplotype shared by ibex and sheep (Figure 3). The resistant  
 520 haplotypes of *T. axei* and *T. colubriformis* were more common than the sensitive  
 521 haplotypes and the most similar sensitive haplotypes are found either in both sheep  
 522 and ibex samples, or only in ibex samples. Both, *T. colubriformis* and *T. vitrinus*  
 523 showed two distinct lineages, separated by  $\geq 10$  mutations. One of the lineages of *T.*  
 524 *vitrinus* was only found in ibex from Belledonne and an ibex from Champsaur (Figure  
 525 3) while the ASVs of the second lineage are found both in ibex and sheep. One of the  
 526 lineages of the *T. circumcincta* was more diverse (Figure 3).

527



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

533 resistant haplotype. The tag above the points indicated the name of the mutation,  
534 based on the codon position and the substitution of the amino acid.

535

536

## Discussion

537

538 Because transhumant sheep and resident Alpine ibex use the same pastures  
539 during the summer, we sought to assess the extent of nematode parasites sharing  
540 between these two host species. Specifically, we investigated the presence of  
541 anthelmintic-resistant nematode strains in sheep and ibex to determine the role of  
542 transhumant sheep in contaminating alpine pastures, and whether ibex may play a  
543 role in the maintenance and circulation of anthelmintic resistant nematodes. We used  
544 a metabarcoding approach based the sequencing of ITS2 and the  $\beta$ -tubulin to  
545 demonstrate that both sheep and ibex were infected by the same gastrointestinal  
546 nematode species and shared anthelmintic-resistant strains, despite the absence of  
547 sheep on alpine pastures for much of the year and therefore a narrow temporal  
548 window of contamination.

549 In line with other studies investigating the gastrointestinal nematodes of sheep  
550 and ibex (Burgess et al., 2012; Gruner et al., 2006; Redman et al., 2019; Zaffaroni et  
551 al., 2000), the most prevalent and abundant species in both host species was  
552 *Teladorsagia circumcincta*. Next, *Trichostrongylus vitrinus* was moderately prevalent,  
553 but not abundant in sheep and ibex nemabiome. In accordance with the climatic  
554 conditions of ibex environment, these two nematode species, as well as *Marshallagia*  
555 spp., are better adapted to cold temperatures than the other nematode species  
556 detected in this study (O'Connor et al., 2006; Zaffaroni et al., 2000). The sheep flocks  
557 originate from French plains and/or the south of France and are driven into mountain  
558 areas in early summer. Consequently, their nemabiome at the time of sampling is  
559 representative of the gastrointestinal nematode communities present in sheep on the  
560 farm, i.e., prior to transhumance. In a similar context, Gruner et al., (2006), observed  
561 a high prevalence of *T. circumcincta* in two of three transhumant sheep flocks at the  
562 beginning of the grazing season in the mountains south of the Alps. Furthermore,  
563 transhumant sheep flocks appear to ingest mainly *T. circumcincta* when grazing in  
564 the mountains, as this parasite remains the dominant species identified in feces and  
565 tracer lambs during the summer (Gruner et al., 2006). Pastoral activity in

566 mountainous areas of France could therefore favor nematode species more adapted  
567 to cool and wet environmental conditions, such as *T. circumcincta* (O'Connor et al.,  
568 2006), compared with sheep grazing on the plains all year round. To confirm this  
569 hypothesis, a study of the variation in the nemabiome of transhumant sheep during  
570 the summer should be undertaken.

571 High relative frequency (>30%) of *H. contortus* was detected in ibex in Belledonne  
572 and Champsaur. In contrast, almost no *H. contortus* was observed in sheep flock  
573 droved in these mountains, raising the possibility that ibex may be contributing to the  
574 reinfection of sheep with *H. contortus*. To our knowledge, this is the first time that  
575 such relative abundance of *H. contortus* is reported in Alpine ibex (see previous  
576 studies based on morphological identification: Carcereri et al., 2021; Marreros et al.,  
577 2012; Zaffaroni et al., 2000). In addition, it should be noted that Alpine ibex were  
578 sampled before a potential contamination by domestic sheep could be detected, i.e.,  
579 before the end of the pre-patent period: time between the infestation and the first  
580 eggs production, and at the end of spring – early summer, i.e., at the start of the  
581 epidemiological period at risk for *Haemonchus contortus* in a high-altitude mountain  
582 area. We can therefore expect higher levels of contaminations in late summer, when  
583 domestic sheep leave mountain pastures. In addition, we cannot exclude that some  
584 laboratory issues might have reduced the apparent prevalence and abundance of *H.*  
585 *contortus* as some samples from sheep have been kept in the fridge at 4°C during 2  
586 to 3 days (including e.g., the sheep samples without *H. contortus* from Belledonne  
587 and Champsaur) which can reduce the proportion of *H. contortus* eggs hatching  
588 (McKenna, 1998).

589 The detection of *H. contortus* raises conservation issues for Alpine ibex as this  
590 nematode species is known to be highly pathogenic to sheep (Taylor et al., 2015).  
591 Infection of a phylogenetically related species, the Pyrenean ibex (*Capra pyrenaica*  
592 *pyrenaica*), with a few thousand of *H. contortus* resulted in severe clinical signs,  
593 including extremely low weight and hemorrhagic anemia (Lavín et al., 1997). In  
594 addition, *H. contortus* may have been involved, along with pneumonia, in the collapse  
595 of the Northern Chamois in the province of Lecco, Italy from November 2000 to  
596 March 2001 (Citterio et al., 2006). As gastrointestinal nematodes can have an impact  
597 on the demographic dynamics of the host population (Acerini et al., 2022; Albery et  
598 al., 2021; Albon et al., 2002), they are suspected of being involved in the low natality  
599 rates observed in the French Alpine ibex populations (Brambilla et al., 2020). While

600 Alpine ibex appears to be fairly resilient to parasite infections (Marreros et al., 2012),  
601 further investigations should be carried out to assess the consequences of  
602 gastrointestinal nematodes infections for ibex at both individual and population level.

603 We detected anthelmintic resistant alleles in 4 out of the 5 nematodes species,  
604 namely *H. contortus*, *T. circumcincta*, *Trichostrongylus axei*, *Trichostrongylus*  
605 *colubriformis*, but not *T. vitrinus*, for which the  $\beta$ -tubulin amplicons have been  
606 sequenced. Both sheep and ibex hosted resistant strains of the 4 nematode species  
607 and only 10 out of 116 ibex did not have resistant strains to any of the nematode  
608 species studied. The benzimidazole resistance was therefore very common in the  
609 studied sheep flocks, in agreement with the situation of sheep farms in Europe (Rose  
610 et al., 2015; Rose Vineer et al., 2020). The presence of anthelmintic resistant  
611 nematodes in ibex is most likely explained by the indirect transmission of resistant  
612 nematodes from sheep to ibex through the environment. The large number of shared  
613  $\beta$ -tubulin ASVs between sheep and ibex and the high overlap between their  
614 nemabiome confirm this scenario (Figure 2c, Figure 3). This is in accordance with  
615 other studies investigating the share of nematode parasites at the interface of wild  
616 and domestic ungulates (Beaumelle et al., 2022; Cerutti et al., 2010; Laca Megyesi et  
617 al., 2019).

618 It is worth noting that feces of ibex were sampled before the arrival of sheep on  
619 pastures. This important result demonstrates that anthelmintic resistant nematodes  
620 can be maintained in mountainous areas from year to year in wild populations of ibex  
621 despite harsh winter environmental conditions, and in the absence of the main  
622 source of parasites during most of the year, i.e., the domestic sheep. The shedding of  
623 eggs from resistant nematodes by ibex prior to the arrival of domestic sheep  
624 suggests the potential role of ibex as a reservoir of anthelmintic resistant nematodes  
625 for other susceptible domestic and wild ungulates. In addition, the position of  
626 resistant mutant strains detected in ibex at the periphery of haplotypes networks  
627 (Figure 3) supported the relatively recent selection of benzimidazole resistance and  
628 the lack of benzimidazole resistant reversion since the resistant strains have been  
629 transmitted to ibex.

630 The 5 nematodes species for which we have studied the resistances seemed to  
631 have different selection dynamics which may reflect the life history traits of species  
632 (Redman et al., 2015). In fact, we detected no resistant allele in *T. vitrinus* and  
633 conversely, the proportion of benzimidazole resistant strains of *T. axei* and *T.*



634 *colubriformis* were high in sheep and in a lesser extent in ibex (Figure 2c). The  
635 resistance proportions of *T. circumcincta* and *H. contortus* were lower compared to *T.*  
636 *axei* and *T. colubriformis*, excepted for the *H. contortus* of the sheep flock of the  
637 Montagne de l'Oule. In this study area, the proportion of resistant strains of *H.*  
638 *contortus* was very high.

639 Consistent with our study, benzimidazole-resistant strains of *T. vitrinus* were rare  
640 in other studies of sheep farms (in the UK, Avramenko et al., 2019, and in Canada,  
641 Queiroz et al., 2020). In contrast, high frequencies of benzimidazole resistance in *T.*  
642 *axei* and *T. colubriformis* (between 40% and 100%) were already reported in sheep;  
643 in UK, *T. axei*: 26-27% and *T. colubriformis*: 53-62% (Avramenko et al., 2019); in  
644 Austria, *T. colubriformis*: 77%-100%, (Hinney et al., 2020); in France, *T. axei*: 63%,  
645 (Palcy et al., 2010). In contrast, Hinney et al., (2020), observed in transhumant sheep  
646 flocks in Austria Alps, a higher mean frequency of the F200Y resistance allele in *T.*  
647 *circumcincta*:  $32.4 \pm 6.8\%$  (mean  $\pm$  standard error of the mean) and *H. contortus*:  
648  $91.9 \pm 3.7\%$ , compared to the sheep flocks of this study (*T. circumcincta*,  $6.6 \pm 3.5\%$   
649 and *H. contortus*,  $69.9 \pm 14.4\%$ , all resistant alleles combined).

650 Several factors are suspected to contribute to interspecific differences in the  
651 selection of resistance strains between nematode species, including specific  
652 reproductive rate, seasonal dynamics, climatic conditions in the location of sheep  
653 farms, anthelmintic strategies, e.g., treatment molecules, timing and rate of  
654 anthelmintic treatments, grazing management and the cost of benzimidazole  
655 resistance (Hodgkinson et al., 2019; Redman et al., 2015). However, the links  
656 between traits of parasites and interspecific variation of resistant acquisition by  
657 gastrointestinal nematodes have not been tested yet (Morgan et al., 2019). Our  
658 results suggested a few clues in relation to the ecology of nematode species.

659 Firstly, nematode species have different abilities to practice hypobiosis, i.e., the  
660 ability to halt embryonic development under environmental constraints (Gibbs, 1986).  
661 *H. contortus* and *T. circumcincta* are known to arrest development more frequently  
662 than *Trichostrongylus* spp. (Langrová et al., 2008), and hypobiotic larvae have been  
663 shown to be less sensitive to drug (Sargison et al., 2007). Secondly, among the  
664 *Trichostrongylus* spp., *T. vitrinus* may have a higher proportion of overwintering  
665 larvae in pastures as this species is more resistant to cold temperature compared to  
666 *T. axei* and *T. colubriformis* (O'Connor et al., 2006). As parasites on pasture are not

667 subject to selection pressure by anthelmintics, they are a source of susceptible  
668 strains.

669 As the proportion of resistant strains is generally lower in ibex compared to sheep,  
670 ibex may have contributed to a dilution effect of resistant strains, i.e., by hosting  
671 susceptible nematodes. However, the role of ibex in the maintenance of a refugia  
672 needs to be investigated by considering the relative number of susceptible strains  
673 deposited by ibex on pasture compared with sheep. Furthermore, it seems that the  
674 role of ibex in the maintenance of a refugia vary according to nematode species. For  
675 example, ibex excrete a lower proportion of *T. axei* eggs than sheep (Figure 2a),  
676 while resistant strains are also dominant in ibex (Figure 2c). In contrast, *T.*  
677 *circumcincta* and *H. contortus* in ibex were more frequently susceptible and  
678 genetically diverse (higher number of ITS2 and  $\beta$ -tubulin ASVs) compared with *T.*  
679 *axei* and *T. colubriformis* ibex (Table 2, Figure 3). As *T. circumcincta* was dominant in  
680 ibex, a refuge of susceptible *T. circumcincta* strains may have been maintained within  
681 ibex and may contribute to limit the spread of resistance in sheep farms. The results  
682 concerning *H. contortus* were variable according to the massifs since ibex from the  
683 two Cerces sites had a low amount of *H. contortus* compared to the two other  
684 populations, i.e., Belledonne and Champsaur.

685 The divergence between massifs can be observed at the community and genetic  
686 level among sheep flocks and ibex populations. Indeed, it was expected that some  
687 differences in nemabiome composition would be observed between the massifs and  
688 sheep flocks, given that sheep and ibex from different areas never meet (R. Papet, C.  
689 Toïgo, E. Vannard, Pers. communication). Furthermore, the sheep flocks come from  
690 different locations and have been subjected to different anthelmintic strategies. For  
691 the ibex, differences in the original population of translocated animals (Gauthier and  
692 Villaret, 1990; Kessler et al., 2022) and potential founder effect – not all parasites  
693 present in the source population were present in the newly established sink  
694 population - may have had a long term impact on the composition of the nemabiome.  
695 For example, the distinct population of *T. vitrinus*, found mainly in ibex in Belledonne,  
696 may had been inherited from the founding ibex population. This highlights that  
697 various reintroductions of ibex in the study area can also influence the composition of  
698 the parasite community. This distinct population of *T. vitrinus* was absent in sheep  
699 grazing in Belledonne, which hosted other strains of *T. vitrinus* and despite several  
700 summers of co-occurrence of sheep and ibex in Belledonne. In is possible that sheep

701 never graze on pastures contaminated by these strains, but this observation may  
702 also be due to a sampling bias as the number of sheep sampled remains low. Within  
703 the same mountain area, few differences have, however, been observed among the  
704 nemabiome of ibex, e.g., between the ibex of the Aiguillette de Lauzet and those of  
705 the Montagne de l'Oule, whereas the sheep flocks host distinct nemabiome  
706 communities.

707 In conclusion, transmissions of gastrointestinal nematodes species, including  
708 resistant nematodes strains, have occurred between sheep and ibex even though the  
709 contact between the two species is limited to the summer period. In this study, we  
710 demonstrated more specifically that ibex can maintain and shed eggs of resistant  
711 gastrointestinal nematodes despite the absence of sheep on pastures for several  
712 months, suggesting a potential role of ibex as a reservoir for these nematodes.  
713 However, the extent to which each host species can influence the nematode  
714 community of the other during the transhumant period remains to be determined. To  
715 this end, it would be useful to analyze the nemabiome of sheep and ibex before and  
716 after the transhumant period. The lower proportion of resistance allele in ibex  
717 nemabiome compared with that of sheep underlines the possibility that ibex could  
718 contribute to the maintenance and circulation of susceptible strains in sheep. Based  
719 on our results, it seems that ibex have helped to limit the spread of anthelmintic  
720 resistance of *T. circumcincta* and *H. contortus* in sheep flocks, by maintaining  
721 parasite refugia not exposed to anthelmintic pressure. As with sheep and roe deer  
722 (Beaumelle et al., 2022), domestic sheep contributes to the modification of the  
723 nemabiome of ibex. This raises concerns about the conservation of the ibex, and the  
724 consequences of strongyles infection in ibex should be investigated. Indeed, ibex is  
725 characterized by low genetic diversity due to the strong demographic decline of this  
726 species, followed by multiple re-introduction (Grossen et al., 2018) and a high genetic  
727 structure of immunity-related loci among populations (Kessler et al., 2022) whereas  
728 both neutral and adaptive genetic diversity are known to have an influence parasite  
729 resistance in ungulates (Portanier et al., 2019).

730

731

## Appendices

732

733 Supplementary data to this article can be found online in MendeleyData (DOI:  
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753

### **Conflict of interest disclosure**

754

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

756

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

757

758  
759 The bioinformatic pipeline, the ASV analysed during the current study and the R  
760 script of statistic analyses are available in MendeleyData (DOI:  
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