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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 β -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 β -tubulin isotype-1, resulting in the disruption of tubulin–microtubule
92 equilibrium (Whittaker et al., 2017). In resistant nematodes, specific mutations of the
93 β -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 β -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 19th 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 20th 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

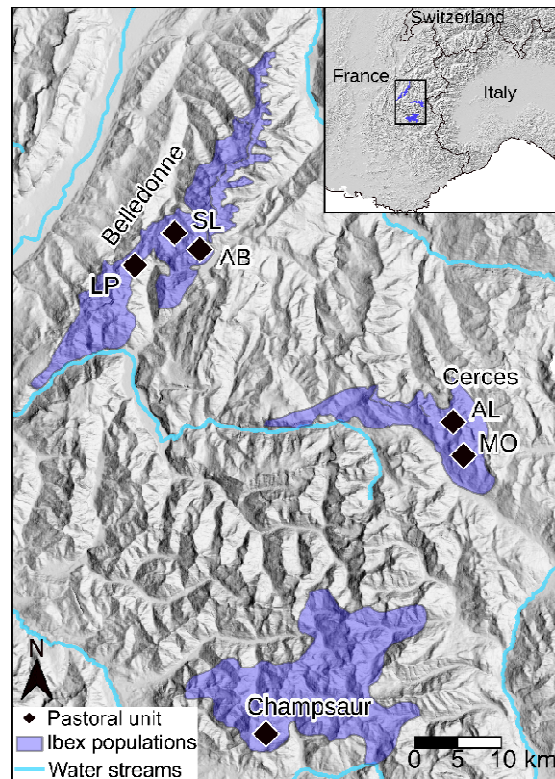
Materials and Methods

180

Study area

181

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 200km², between 1410m and 3100m on 120km² and between
213 1320m and 3550m on 280km², 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
Cerces mountain			
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]
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 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 ± 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 H₂O) 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 β -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 β -tubulin isotype 1) based on their initial concentration
310 and in proportion according to the following ratio: ITS2 50%, β -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 β -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. 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 β -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 β -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 β -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 β -tubulin isotype 1 ASVs were aligned to one
347 of the β -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 β -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 β -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

400 *Parasite material*

401

402 The median number of eggs of strongyles per gram of feces were lower in sheep
403 (7.5[0,148]_{95%IQR}; n = 90) than in ibex (15[0,163]_{95%IQR}; 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

412 *Diversity of gastrointestinal nematodes in sheep and ibex*

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 *Teladorsagia circumcincta* was the most prevalent nematode species and was
421 detected in 85% of samples (90%, n=127/141 ibex and 71%, n=39/56 sheep),
422 followed by *Trichostrongylus vitrinus*, 63% (73%, n=103/141 ibex and 36%, n=20/55
423 sheep) and *Haemonchus contortus*, 56% (70%, n=98/141 ibex and 22%, n=12/55
424 sheep) (Table 2, Figure S4). *Nematodirus* spp. and *Ostertagia leptospicularis* were
425 the rarest species and were detected in only 2 samples and 1 sample, respectively
426 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 β -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			β -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%]_{95CI} in sheep feces and 2% [1%;2%]_{95CI} 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%]_{95CI}) than in Belledonne (mean RRA: 4% [2%; 6%]_{95CI}). Likewise, *Marshallagia*
 449 spp. was more frequent in ibex feces in Cerces and Champsaur mountains (mean
 450 RRA: 11% [6%;15%]_{95CI}) than in ibex feces in Belledonne (0.4% [-0.2%; 1%]_{95CI}). 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).

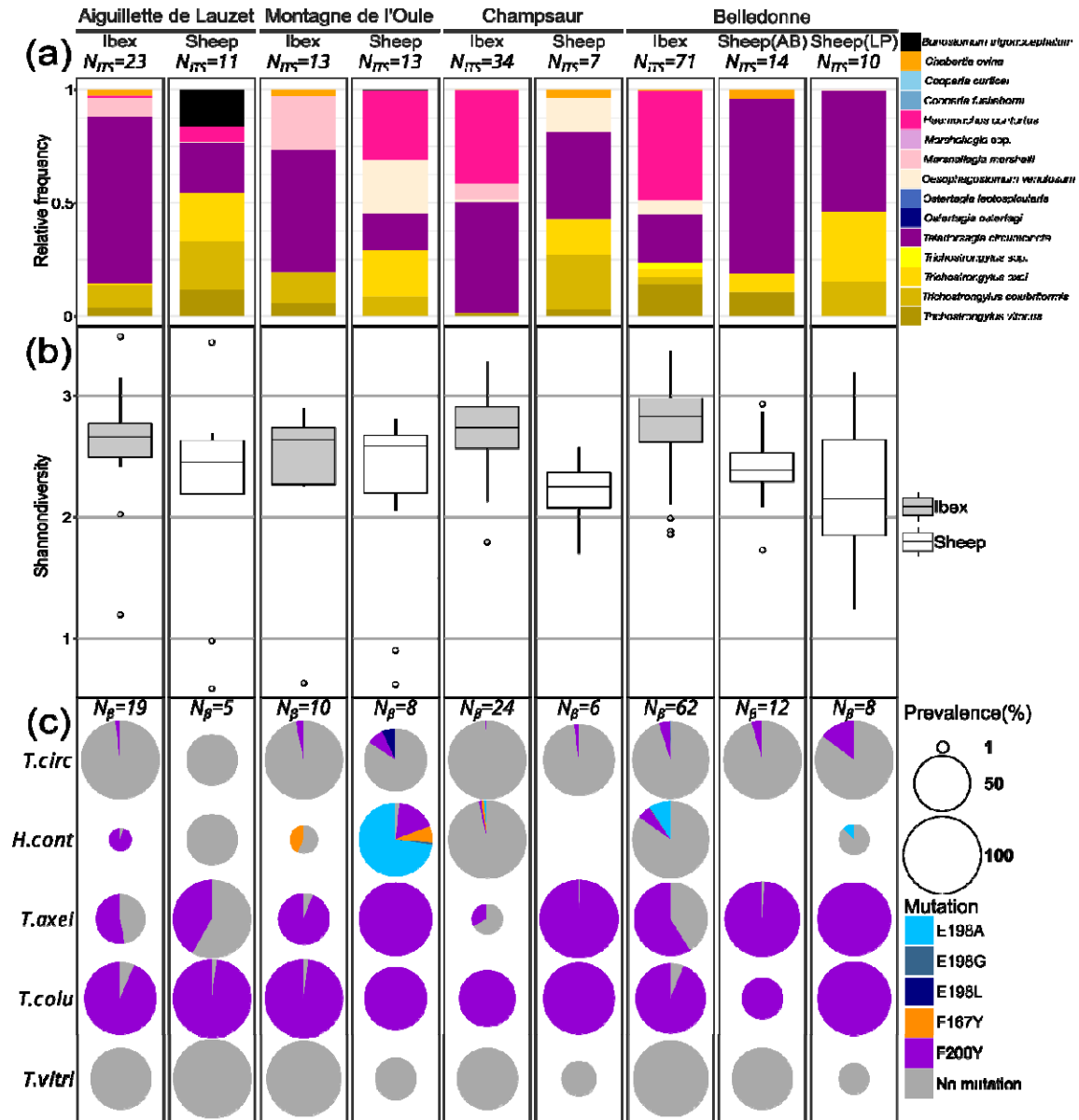
457

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 R² are reported with the
 462 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

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 β -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 β -tubulin
 471 isotype 1 (N_{β} ; 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 β -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]_{95CI}; *T. circumcincta*: 4% [3;6]_{95CI}; *T. axei*: 70% [63;78]_{95CI}; *T. colubriformis*:
496 96% [93;99]_{95CI}). The resistant RRA is generally lower in ibex compared to sheep (β
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

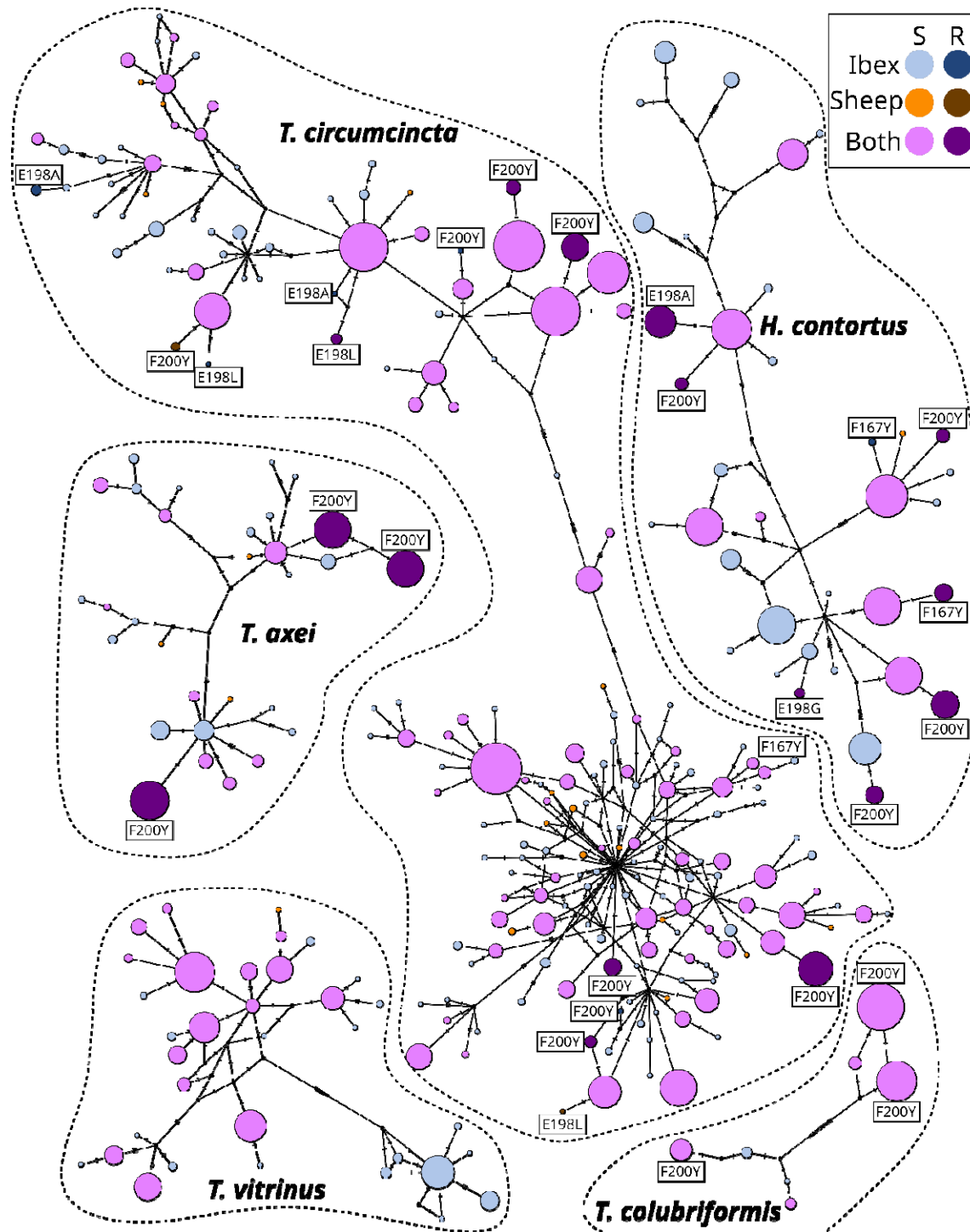
510 reference) are reported. Parameter estimates with standard error (SE) are reported
 511 with the corresponding *z-value* (*z-val*) and *p value* (*P*). AL: Aiguillette de Lauzet; MO:
 512 Montagne de l'Oule; Ch: Champsaur; Hc: *Haemonchus contortus*; Ta:
 513 *Trichostrongylus axei*; Tcol: *Trichostrongylus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

Best model selected	Variables	Parameter estimate ± SE	<i>z</i> -val	<i>P</i>
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%) β -tubulin isotype 1 haplotypes and 238
 516 (55%) β -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 ≥ 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



528

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532

Figure 3 : Median joining network of β -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 β -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 β -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 β -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 β -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).

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731

Appendices

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733 Supplementary data to this article can be found online in MendeleyData (DOI:
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Conflict of interest disclosure

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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:
761 10.17632/cm97cg87d6.1).

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