

1 **UNEXPECTED *ECHINOCOCCUS MULTILOCULARIS* INFECTIONS IN SHEPHERD DOGS**
2 **AND WOLVES IN SOUTH-WESTERN ITALIAN ALPS: A NEW ENDEMIC AREA?**

3

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23 **Author contributions.** Massolo ideated the study, the sampling design and wrote the first draft of
24 the MS; DV collected samples in the field and carried classic and molecular parasitology analyses;
25 MW and TR supervised molecular analyses and contributed to writing the manuscript; SdA and SC
26 carried out confirmatory molecular analyses and critically contributed to the manuscript; Meriggi
27 coordinated and supervised field work; ET and SM collected samples and coordinated field work;
28 LZ, CBB and MO carried out classic molecular analyses; AC critically contributed to the manuscript;
29 FM supervised the classic parasitology analyses and wrote the more general parasitology
30 component of the MS.

31

32 **HIGHLIGHTS**

- 33 **1.** We detected *Em* infections in wolves and shepherd dogs 130 km south of the southern-
34 most case reported so far in the European Union.
- 35 **2.** *Em* infections in wolves and dogs in this area may indicate a range expansion and the
36 establishment of a new endemic area.
- 37 **3.** This area is along an ecological corridor connecting the Alps to the Apennines, with the
38 potential for further expansion.
- 39 **4.** It is priority to implement surveillance on the local fox population, and inform practitioners
40 of the increased risk.

41

42 ***Running title:*** New endemic area for *E. multilocularis* in Italy.

43

44

45 **ABSTRACT**

46 The European distribution of *Echinococcus multilocularis* has been reported to be expanding east
47 and north, whereas its southern limits are deemed stable. During a study on *Echinococcus*
48 *granulosus* s.l. infections in wolves and dogs in the Italian Maritime Alps, we unexpectedly
49 detected the presence of *E. multilocularis* eggs in four fecal samples from at least two shepherd
50 dogs, and in five wolf fecal samples. This finding, in an area about 130 km south of the
51 southernmost *E. multilocularis* report in the Alps, may suggest a rapid expansion southward. While
52 infections in foxes are currently being investigated, these data seem to indicate the potential for a
53 new *E. multilocularis* endemic area. If this will be confirmed, the implementation of surveillance
54 programs in wild and domestic canids and preventative measures will become a priority.

55

56 **Keywords:** *Echinococcus multilocularis*; Italy; parasite range expansion; *Canis lupus*; domestic dog;
57 alveolar echinococcosis.

58 **INTRODUCTION**

59 *Echinococcus multilocularis*, a tapeworm with an indirect life cycle involving mammalian predators
60 as definitive hosts (primarily wild or domestic canids; DH hereafter) and mammalian prey species as
61 intermediate hosts (primarily small rodents; IH hereafter; reviewed in (!!! INVALID CITATION !!!
62 (Romig et al., 2017))), is the etiological agent of alveolar echinococcosis (AE hereafter), the third
63 most relevant human foodborne parasitic disease worldwide (FAO/WHO, 2014). Once mature, the
64 adult worms, attached to the intestinal tract of the DHs, release in the host intestinal lumen their
65 last proglottids with hundreds of embryonated eggs that will be later excreted in the environment
66 within the host feces. Once ingested by a competent IH, or by people (as dead-end host), the eggs
67 hatch and release larvae (oncospheres) in the intestinal tract of the host that pass through the
68 intestinal wall into the blood vessels, and from there to the target organs (almost exclusively the
69 liver). There the oncospheres will develop into metacestodes that will replicate asexually producing
70 thousands of infectious larvae (protoscoleces). When the infectious IH is preyed upon by a
71 competent DH, the protoscoleces evaginate, attach to the wall of the small intestine and develop
72 into adults. The asexual multiplication phase of the metacestode creates very severe clinical
73 conditions, due to an infiltrating tumor-like process that damage the tissues and will eventually
74 invade other organs through a metastasis-like process (Kern et al., 2017). Human AE is estimated to
75 affect more than 18,000 new patients worldwide every year (Torgerson et al., 2010), with about
76 150-200/year expected in central Europe alone, and an incremental trend in the last decades
77 (Vuitton et al., 2015). Human AE is deadly if untreated, and still highly pathogenic with a fatality of
78 16% despite recent advances in treatment (Kern et al., 2017). In Europe the parasite is distributed
79 mostly in its central and eastern part, extending into northern France, southern Scandinavia
80 (Denmark, southern Sweden) and parts of the Balkan peninsula (Combes et al., 2012; Deplazes et
81 al., 2017; EFSA, 2015; Oksanen et al., 2016; Umhang et al., 2016).

82 At present, the parasite is deemed to be expanding north and east, but its southern limits, at least
83 in the Alpine region, are described as stable (Conraths and Deplazes, 2015) and some authors
84 suggested that the limited distribution of *Microtus arvalis* in southern slopes of the Alps may play a
85 role in limiting *E. multilocularis* distribution in Switzerland (Guerra et al., 2014). Recent findings of
86 *E. multilocularis* infections in foxes from Southern France in the Hautes Alpes Department (05) seem
87 to indicate a possible expansion southward (Combes et al., 2012; Umhang et al., 2016).
88 In Italy, the only reports of *E. multilocularis* infections have been in the eastern Alps in the Trentino-
89 Alto Adige Region (EFSA, 2015) where multi-locus microsatellite analysis suggested an
90 autochthonous focus of the parasite (Casulli et al., 2009), and where a recent meta-analysis based
91 on 26 papers indicated a prevalence in foxes between 0.5 and 2.9 (Oksanen et al., 2016).

92

93 **MATERIAL AND METHODS**

94 From June to November 2017, we conducted a fecal survey of taeniid cestodes in wild and
95 domestic canids in a protected area (Parco Regionale delle Alpi Liguri) of the Southern Italian Alps
96 in the Imperia Province (Liguria region), about 25 km from the Mediterranean coast in Italy (Fig. 1).
97 The area is located in north-western Italy and connects the Apennines with the Maritime Alps
98 (44°10'N 8°05'E; 6041 ha). The elevation ranges from 0 to 2,200 m a.s.l. Chestnut and beech
99 woods are the predominant forests. Meso-fauna includes wolf *Canis lupus*, fox *Vulpes vulpes*, and
100 dogs *Canis lupus familiaris* as predators, and roe deer *Capreolus capreolus*, chamois *Rupicapra*
101 *rupicapra*, and the wild boar *Sus scrofa*. Livestock amounts to more than 3,200 cattle, 1,200 sheep
102 and goats, and ca 50 horses. Potential IH species for *E. multilocularis* includes *Arvicola scherman*,
103 *Chionomys nivalis*, *Microtus multiplex*, *Microtus savii*, *Myodes glareolus*, *Apodemus alpicola*,
104 *Apodemus flavicollis*, *Apodemus sylvaticus*, *Mus musculus* along with *Rattus norvegicus* and *Rattus*
105 *rattus*.

106 We identified 10 standard pathways (4.3 ± 0.27 km) that were walked on a monthly basis to locate
107 and collect feces of wolves and domestic dogs, particularly those owned by local shepherds.
108 In order to correctly discriminate wolf feces to those of dogs, molecular host identification was
109 performed purifying the genomic DNA with the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden,
110 Germany) and analyzing the 281 bp sequence of the mtDNA control region (Sindičić et al., 2011).
111 Samples were stored at -80°C for at least 3 days to deactivate the eggs (Veit et al., 1995), then
112 kept at -20°C until subsequent analyses.
113 The samples were first screened for taeniid eggs using ZnCl_2 flotation followed by sieving (Mathis
114 et al., 1996). Single eggs were separated and molecularly characterized using partial sequences of
115 the *nad1* mtDNA gene as marker (Hüttner et al., 2008)(Tab. 1). Egg samples which yielded no
116 positive result after *nad1* PCR were further analyzed targeting sequences of the *cob* gene (Hüttner
117 et al., 2008)(Tab. 1). Readable sequences of partial *nad1* and *cob* were aligned using MEGA7
118 (Kumar et al., 2016) and compared to GenBank retrieved homologous sequences of *Echinococcus*
119 *multilocularis*, *E. granulosus*, *E. ortleppi*, *E. canadensis*, *Taenia hydatigena*, *T. krabbei* and *T. ovis*,
120 according to availability.

121

122 **RESULTS AND DISCUSSION**

123 We collected a total of 183 fecal samples. Definitive host species identification by sequencing part
124 of the mtDNA control region confirmed field observations for all the *E. multilocularis*-positive dog
125 fecal samples, while one wolf fecal sample (IM WLF 79) out of six *E. multilocularis*-positives turned
126 out to be of a dog so we ended up with 151 feces of wolves and 32 of dogs. We then could screen
127 152/183 feces (wolf, 120; dog, 32; the rest were discarded from the analyses as they were either
128 too small to allow for both diet analysis and parasitology, or too dry and old) and detected
129 Taeniidae eggs in 11.66% of wolf feces (14/120) and 15.62% of dog feces (5/32).

130 We isolated 10 to 20 single taeniid eggs from 14 wolf fecal samples and from 5 dog fecal samples.
131 Four out of five dog fecal samples contained *E. multilocularis* eggs along with other Taeniidae
132 infections (*Taenia hydatigena*, *T. krabbei*, *T. ovis*; Tab. 2), whereas only 5 out of the 14 wolf fecal
133 samples contained *E. multilocularis* eggs, and showed coinfection with *Taenia* spp. and *E. orteppi*
134 in two samples (see sequences alignments for positive specimens of *E. multilocularis* at partial *cob*
135 Fig.2a and 2b; and for positive specimens of *E. orteppi* at partial *nad1*, Fig. 2c).

136 Importantly, some fecal samples of both wolf and dog were from the same individuals, but we are
137 sure that the dog samples came from at least two different individuals belonging to two different
138 shepherds, and not allowed to roam at night. No travel history that may justify allochthonous
139 infections was reported for these shepherd dogs.

140 Although we cannot exclude that some of the wolf fecal samples were from the same animal,
141 previous studies have indicated the presence of at least two or more distinct wolf packs in the
142 areas we investigated (Imbert et al., 2016; Marucco and Avanzinelli, 2017), so we believe that the
143 positive wolf fecal samples are from more than one individual. Individual wolf genotyping from
144 our fecal samples was not conducted because of lack of sufficient fecal matter after the repeated
145 molecular testing.

146 Whereas the *Taenia* spp. infections were known to be common in these areas (Gori et al., 2015),
147 the detection of *E. orteppi* (G5) is unexpected. This is the first report of G5 in Italy in wolves, and
148 in general in DHs in this country. The cycle of this parasite is mainly cattle–dog, but this species
149 has also been identified in other IHs such as buffaloes, pigs, sheep and humans as dead-end hosts
150 (Dinkel et al., 2004; Romig et al., 2017). *Echinococcus orteppi* was reported for the first time in
151 Italy by Casulli and colleagues (Rinaldi et al., 2008) in one bovid, and infections without fertile
152 cysts of the cattle strain were reported in Campania (Rinaldi et al., 2008). Definitely, considering
153 the potential for zoonotic infections, further investigation in dogs and wolves is to be

154 implemented in this area to understand which wild and domestic IH species are involved in the
155 maintenance of the parasite in the environment.

156 Finally, but more importantly, the finding of *E. multilocularis* in both dogs and wolves was not only
157 unprecedented, but also surprising and remarkable, given its high zoonotic potential. In the last
158 years, a screening of *E. multilocularis* infections in fox carcasses was carried out (Magi et al., 2015),
159 along with a screening of gastrointestinal parasites in wolves (Gori et al., 2015), but no *E.*
160 *multilocularis* infections were detected.

161 In our study area, there are several species of potentially competent IHs, and both dogs and
162 wolves are known to host *E. multilocularis* (Romig et al., 2017). A previous study on wolf diet
163 indicated predation upon small mammals with a frequency up to 7.6% in the same area (Imbert et
164 al., 2016). Similarly, free ranging shepherd dogs are likely to be predators of these small mammals
165 as well, although we do not expect that to be frequent. Despite that, it is not very likely that a
166 stable *E. multilocularis* population could be maintained without the involvement of a DH species
167 with a diet more focused on IHs such as the red fox. With one exception (China, Tibetan plateau
168 (Moss et al., 2013; Vaniscotte et al., 2011), there is no evidence that wolves and dogs may sustain
169 the cycle.

170 For this reason, we have already started a collection of fox feces and organizing an opportunistic
171 fox carcass interception in coordination with the local animal health agencies, but more in-depth
172 studies are needed to assess the actual extent of the current distribution of *E. multilocularis* in this
173 region and the possible origin of infections. Likely, a larger screening of foxes along the Maritime
174 Alps on both sides of the border (France and Italy) will be required. Moreover, since *E.*
175 *multilocularis* positive dogs are expected to be present only in high endemic areas, our finding
176 poses a potential threat for public health, and a screening of *Echinococcus* infections in dogs is
177 strongly recommended.

178

179 This is the southern-most report of *E. multilocularis* in Italy, and the southern-most in Europe
180 beside the most recent findings in Croatia and Serbia (Beck et al., 2018; Lalošević et al., 2016), and
181 an older record from the European part of Turkey (Deplazes et al., 2017). This infection is likely
182 due to a south-eastward expansion of the current *E. multilocularis* range in France.

183 Being a reportable disease, we recommend to start an epidemiological surveillance program to
184 monitor the distribution of *E. multilocularis* in the Maritime Alps, along with an alert to the public
185 health and veterinary professionals to warn about the possibility of AE in people and animals in
186 the area.

187 The study of marginal populations of *E. multilocularis* offers a unique opportunity to explore the
188 emergence of parasites with complex life cycles at the edge of their distribution, and explore the
189 role of the host community in the establishment of new endemic areas. Moreover, monitoring these
190 marginal populations is also of paramount importance to detect parasite range expansion and
191 develop proper evidence-based surveillance strategies and preventative measures.

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200 Medicine of the University of Zurich, CH), Prof. Patrick Giraudoux (Chrono-environment lab, UMR

201 UFC/CNRS 6249, INRA, University of Franche-Comté, Besançon Cedex, FR) for information on the
202 distribution of the southern locations of *Echinococcus multilocularis*.

203

204 **LIST OF TABLES**

205 **Table 1.** Primer pairs used for PCR to molecularly characterize *Echinococcus multilocularis* in canid
206 fecal samples from the Imperia area in Liguria, Italy, in 2017.

207 **Table 2.** Molecular analysis results conducted on eggs harvested from fecal samples of wolves
208 (WLF; upper table) and dogs (DOG; lower table) collected from June to November 2017 in
209 a mountainous area in the Alps of the Imperia Province, Italy. In table are reported the
210 total number of eggs harvested in each sample, the number of eggs positive (+ve) to *nad1*
211 and *cob* and the corresponding sequencing results.

212

213 **LIST OF FIGURES**

214 **Figure 1.** Locations of wolf (blue dots) and dog (orange dots) fecal samples positive to
215 *Echinococcus multilocularis* collected during a survey on *Echinococcus* spp. carried out
216 from June to November 2017 in a mountainous area in the Alps of the Imperia Province,
217 Italy. In the map are also reported the southernmost reports of *Echinococcus*
218 *multilocularis* (*E. multilocularis*) to date in Europe (France, Drs. Boué and Umhang, pers.
219 communication; North-Eastern Italian Alps, Croatia, as in (Beck et al., 2018)). Two dog
220 fecal samples were collected from the same pasture and are represented by a single dot
221 (noted as 2x).

222 **Figure 2.** Multiple alignment of partial mitochondrial *cob* (124bp) from three specimens identified
223 as *Echinococcus multilocularis* analyzed in the present paper (the first three input
224 sequences) with: **(a)** six *E. multilocularis* sequences retrieved from GenBank after

225 comparison by Local Alignment Search Tool BLAST; **(b)** five sequences referred to
226 *Echinococcus granulosus* (Eg, EgG1), *E. ortleppi* (EgG5), *E. canadensis* (EgG6-7) and *Taenia*
227 *hydatigena* (Thy) retrieved from GenBank. **(c)** Multiple alignment of partial mitochondrial
228 *nad1* (139bp) from three specimens identified as *Echinococcus ortleppi* analyzed in the
229 present paper (the first three input sequences) with sequences retrieved from GenBank
230 belonging to other representatives of *E. ortleppi*, *E. granulosus*, *E. canadensis*, *E.*
231 *multilocularis*, *T. krabbei*, *T. ovis* and *T. hydatigena*. Dots indicate identity with nucleotide
232 of the first sequences listed.

233 **Table 1**

Target genes	Primers for the first PCR (5'-3') ¹	Primers for the second PCR (5'-3')
<i>nad1</i> (mtDNA) ²	F:	
	TGTTTTTGAGATCAGTTCGGTGTG	F: CAGTTCGGTGTGCTTTTGGGTCTG
	R:	R: GAGTACGATTAGTCTCACACAGCA
	CATAATCAAACGGAGTACGATTAG	
<i>cob</i> (mtDNA) ²	F:	
	TTATGCTATACTTCGGTGTATTA	F: TCGGTGTATTAATTCGAAGATTG
	R:	R: GATGACAACCACCCAAATAAGTC
	ATAAGGATACTCCGGATGACAAC	

234 ¹F, forward primer; R, reverse primer.

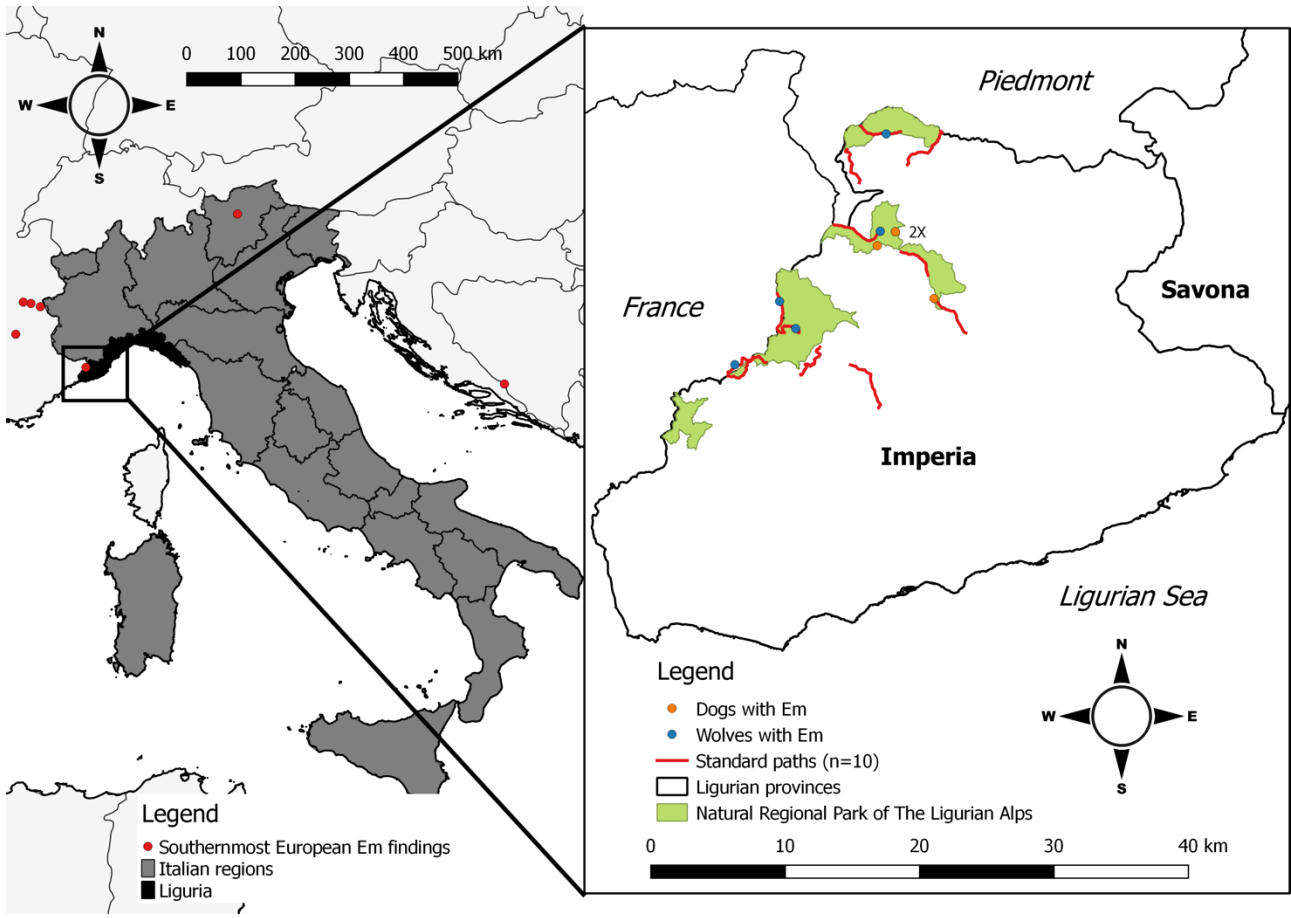
235 ²Primers designed by (Hüttner et al., 2008).

236

ID	N Taeniid Eggs extracted	<i>nad1</i>		<i>cob</i>	
		N +ve eggs	sequencing results	N +ve eggs	sequencing results
IM WLF 42	7	0	NA	2	42.4 <i>Echinococcus multilocularis</i> 42.7 <i>Taenia hydatigena</i>
IM WLF 88	10	6	L 88.2 <i>Echinococcus orteppi</i> L 88.4 <i>Echinococcus multilocularis</i> L 88.5 <i>Echinococcus orteppi</i> L 88.7 <i>Taenia krabbei</i> L 88.8 <i>Echinococcus orteppi</i> L 88.10 <i>Taenia krabbei</i>	0	NA
IM WLF 94	10	6	94.1 <i>Taenia hydatigena</i> 94.2 <i>Taenia hydatigena</i> 94.3 <i>Taenia hydatigena</i> 94.4 <i>Taenia ovis</i> 94.5 <i>Taenia hydatigena</i> 94.8 <i>Taenia hydatigena</i>	1	94.10 <i>Echinococcus multilocularis</i>
IM WLF 107	10	0	NA	3	107.1 <i>Taenia krabbei</i> 107.2 <i>Taenia hydatigena</i> 107.4 <i>Echinococcus multilocularis</i>
IM WLF 143	10	8	143.1 <i>Taenia hydatigena</i> 143.2 <i>Taenia hydatigena</i> 143.3 <i>Echinococcus multilocularis</i> 143.4 <i>Taenia hydatigena</i> 143.5 <i>Taenia hydatigena</i> 143.7 <i>Echinococcus orteppi</i> 143.8 <i>Taenia hydatigena</i> 143.9 <i>Echinococcus orteppi</i>	0	NA
IM WLF 79 (DOG*)	7	3	79.1 <i>Taenia ovis</i> 79.2 <i>Taenia ovis</i> 79.6 <i>Taenia ovis</i>	4	79.3 <i>Taenia ovis</i> 79.4 <i>Echinococcus multilocularis</i> 79.5 <i>Echinococcus multilocularis</i> 79.7 <i>Taenia ovis</i>
DOG 11	9	3	C 11.2 <i>Taenia hydatigena</i> C 11.7 <i>Echinococcus multilocularis</i> C 11.8 <i>Taenia hydatigena</i>	0	NA
DOG 14	10	4	14.6 <i>Taenia hydatigena</i> 14.7 <i>Echinococcus multilocularis</i> 14.9 <i>Taenia hydatigena</i> 14.10 <i>Taenia hydatigena</i>	1	14.8 <i>Echinococcus multilocularis</i>
DOG 30	10	2	30.3 <i>Taenia hydatigena</i> 30.9 <i>Echinococcus multilocularis</i>	0	NA

239 *Initially classified as wolf.

240 **Figure 1**



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Figure 2a

CANE14_8_cob_Em	GGG	GGT	TGT	TGT	TGA	TTT	TAT	CTT	TTA	TTT	TTT	TCT	TAT	GAG	TAC	CAA	[48]
IMCL79_5_Em	[48]
IMCL94_10_Em	[48]
MF370870_Em	[48]
MF370869_Em	[48]
AB461400_Em	[48]
AB374426_Em	[48]
AB510026_Em	.T.C	[48]
AB461402_Em	.T.C	[48]
CANE14_8_cob_Em	CTG	AGG	GTG	GCA	CTA	GTG	TTT	ATA	GAG	TTT	GGC	GTC	AGG	TTA	ATT	TTT	[96]
IMCL79_5_EmC.	[96]
IMCL94_10_Em	[96]
MF370870_Em	[96]
MF370869_Em	[96]
AB461400_Em	[96]
AB374426_Em	[96]
AB510026_Em	[96]
AB461402_Em	[96]
CANE14_8_cob_Em	GGT	TAG	TTG	TTA	GTT	TGT	TTT	TGT	CAT	T							[124]
IMCL79_5_Em	[124]
IMCL94_10_Em	[124]
MF370870_Em	[124]
MF370869_EmG	[124]
AB461400_EmA.	[124]
AB374426_EmA.	[124]
AB510026_EmA.	[124]
AB461402_EmA.	[124]

Figure 2b

CANE14_8_cob_Em	GGG	GGT	TGT	TGT	TGA	TTT	TAT	CTT	TTA	TTT	TTT	TCT	TAT	GAG	TAC	CAA	[48]
IMCL79_5_Em	[48]
IMCL94_10_Em	[48]
AB786664_Eg	.T.C	..CA	.G.TC	.G.	..AG.	[48]
AF297617_EgG1	.T.C	..CA	.G.TC	.G.	..AG.	[48]
NC_011122_EgG5	.C.A.G	.G.CGG.	.G.	..AT.	[48]
MH300954_EgG6	.T.G	.G.CGA.	.G.T.	[48]
MH301022_EgG7	.T.G	.G.CGA.	.G.T.	[48]
GQ228819_ThyA.	..AG	.A.AG	CT.T	.A.A.	.G.	..A	..T.	..T.	[48]
CANE14_8_cob_Em	CTG	AGG	GTG	GCA	CTA	GTG	TTT	ATA	GAG	TTT	GGC	GTC	AGG	TTA	ATT	TTT	[96]
IMCL79_5_EmC.	[96]
IMCL94_10_Em	[96]
AB786664_Eg	.A.	.A.GT	..G.G.	..G.	[96]
AF297617_EgG1	.A.	.A.GT	..G.G.	..G.	[96]
NC_011122_EgG5A.	..C.	..GTA.AA.A	..A.	[96]
MH300954_EgG6T.GTA.AA	..A.	[96]
MH301022_EgG7T.GTA.AA	..A.	[96]
GQ228819_Thy	..A	GTA	A..	..TTA.	..C.	AT.	..G.AT	..G.	..A.	[96]
CANE14_8_cob_Em	GGT	TAG	TTG	TTA	GTT	TGT	TTT	TGT	CAT	T							[124]
IMCL79_5_Em	[124]
IMCL94_10_Em	[124]
AB786664_Eg	.A.	..AG.A.G.	..G.	[124]
AF297617_EgG1	.A.	..AG.A.G.	..G.	[124]
NC_011122_EgG5AG.A.	..C.GC	[124]
MH300954_EgG6GAA.A.G.	..G.	[124]
MH301022_EgG7GAA.A.G.	..G.	[124]
GQ228819_Thy	.A.	..GATA.T.	..T.	..T.	[124]

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Figure 2c

IMCL143_7_nad1_G5	TTA	GGT	TTG	AGG	CTT	GTT	TTA	TGT	GTG	TGG	TTA	TTT	TTT	GTG	CTT	TGT	[48]	
IMCL113_7nad1_G5C	...	[48]	
ALPECL37_2nad1_G5	[48]	
MG271922_Eortleppi	[48]	
LC167081_Eortleppi	T	[48]	
KT363810_Eortleppi	[48]	
AJ237636_Eortleppi	[48]	
AB979274_Eortleppi	[48]	
EU704122_EmAAT	[48]	
AB668376_EmAAT	[48]	
KT988120_EgranulosusCA	[48]	
KJ663949_EgranulosusACA	[48]	
KX010880_EcanadensisCA	[48]	
EU544632_Tkrabbei	.AA	.TA	C	..G	.A	[48]	
MH287111_Tovis	.AA	.T	.C	.A	C	..A	...	[48]	
JN831284_Thydatigena	.AT	CGT	..G	..A	[48]	
AJ277409_Thydatigena	.AT	CGT	..G	..A	[48]	
AJ277408_Thydatigena	.AT	CAT	..G	..A	[48]	
IMCL143_7_nad1_G5	GTT	GTT	GTA	GGT	ATA	ATT	TAA	TTG	ATT	TTT	ATC	ATA	GTT	ACT	GGT	GGA	[96]	
IMCL113_7nad1_G5	[96]	
ALPECL37_2nad1_G5C	[96]	
MG271922_Eortleppi	[96]	
LC167081_EortleppiA	[96]	
KT363810_Eortleppi	[96]	
AJ237636_Eortleppi	[96]	
AB979274_Eortleppi	.C	[96]	
EU704122_Em	...	ACGT	GT	..A	..A	[96]	
AB668376_Em	...	ACGT	GT	..A	..A	[96]	
KT988120_EgranulosusGTA	[96]	
KJ663949_EgranulosusGTT	[96]	
KX010880_EcanadensisGTA	[96]	
EU544632_Tkrabbei	.C	A	A	..T	...	G	..GG	..GC	T	T	TG	..CA	GT	...	TAT	[96]
MH287111_Tovis	..A	ATGG	..G	T	T	T	..CG	GT	TAT	[96]
JN831284_Thydatigena	..G	A	..T	..TGG	A	..T	T	TG	..G	..T	TCT	[96]
AJ277409_Thydatigena	..G	A	..T	..TGG	A	..T	T	TA	..G	..T	TTT	[96]
AJ277408_Thydatigena	..G	A	..T	..TGG	A	..T	T	TA	..G	..T	TTT	[96]
IMCL143_7_nad1_G5	GTT	GGT	TAT	TAT	TAC	CAT	TAA	TTT	ATG	GGT	TAT	TCT	TGG	TGT	G		[139]	
IMCL113_7nad1_G5T	[139]	
ALPECL37_2nad1_G5T	[139]	
MG271922_EortleppiT	[139]	
LC167081_EortleppiT	[139]	
KT363810_EortleppiT	C	C	[139]	
AJ237636_EortleppiTT	[139]	
AB979274_EortleppiT	[139]	
EU704122_Em	.C	T	..G	..G	..TGA	..G	..T	[139]	
AB668376_Em	.C	T	..G	..G	..TGA	..G	..T	[139]	
KT988120_EgranulosusAC	[139]	
KJ663949_EgranulosusAC	[139]	
KX010880_EcanadensisC	[139]	
EU544632_Tkrabbei	CGG	TTA	..GG	..T	..T	..T	GTT	..G	TGA	TT	...	ATG	..AA	..A	[139]	
MH287111_Tovis	CAA	TTG	..GA	..T	..T	..G	GTG	..G	TA	TT	...	AT	..A	..T	[139]	
JN831284_Thydatigena	C	..GG	..TG	..G	T	..T	TAC	..T	AT	..A	..A	..	[139]	
AJ277409_Thydatigena	C	..GG	..T	..G	..G	..G	T	..T	TAC	..T	AT	..A	..A	..	[139]	
AJ277408_Thydatigena	C	..GG	..T	..G	..G	..G	T	..T	TAC	..T	AT	..A	..A	..	[139]	

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