

Additional file 2.

Table: BLASTn search results for HRM_Cer *Ovis* spp. amplicons, showing the lack of a reliable amplicon species/subspecies identity assignment given the high (100-99%) and equal identity score resulting for different *Ovis* spp..

HRM_Cer amplicon from engorged female from mouflon	BLASTn: Sequences producing significant alignments	Max score	Total score	Query cover	e-value	Ident %
IRH000213	<i>Ovis orientalis</i> breed Asian mouflon	302	302	100	1E-78	100
	<i>Ovis aries</i> breed Jingzhong	302	302	100	1E-78	100
	<i>Ovis aries</i>	302	302	100	1E-78	100
	<i>Ovis aries</i>	302	302	100	1E-78	100
	<i>Ovis aries musimon</i>	302	302	100	1E-78	100
HRM_Cer amplicons from questing nymphs						
IRQ52013_Mez	<i>Ovis aries musimon</i>	300	300	100	5E-78	100
	<i>Ovis aries</i>	298	298	99	2E-77	100
	<i>Ovis aries</i>	298	298	99	2E-77	100
	<i>Ovis aries</i>	298	298	99	2E-77	100
	<i>Ovis orientalis</i> breed Asian mouflon	294	294	100	2E-76	99
IRQ52513_Mez	<i>Ovis orientalis</i> breed Asian mouflon	303	303	100	4E-79	100
	<i>Ovis aries</i> breed Jingzhong	303	303	100	4E-79	100
	<i>Ovis aries</i>	303	303	100	4E-79	100
	<i>Ovis aries</i>	303	303	100	4E-79	100
	<i>Ovis aries musimon</i>	303	303	100	4E-79	100
IRQ079212_A_Ala	<i>Ovis orientalis</i> breed Asian mouflon	296	296	100	6E-77	99
	<i>Ovis aries</i> breed Jingzhong	296	296	100	6E-77	99
	<i>Ovis aries</i>	296	296	100	6E-77	99
	<i>Ovis aries</i>	296	296	100	6E-77	99
	<i>Ovis aries musimon</i>	296	296	100	6E-77	99
IRQ15013_Vge	<i>Ovis orientalis</i> breed Asian	302	302	100	1E-78	100
	<i>Ovis aries</i> breed Jingzhong	302	302	100	1E-78	100
	<i>Ovis aries</i>	302	302	100	1E-78	100
	<i>Ovis aries</i>	302	302	100	1E-78	100
	<i>Ovis aries musimon</i>	302	302	100	1E-78	100
IRQ059412_Pin	<i>Ovis ammon hodgsoni</i>	303	303	100	4E-79	100
	<i>Ovis aries ophion</i>	298	298	100	2E-77	99
	<i>Ovis orientalis</i> breed Asian mouflon	298	298	100	2E-77	99
	<i>Ovis aries</i> breed Jingzhong	298	298	100	2E-77	99
	<i>Ovis aries</i>	298	298	100	2E-77	99
	<i>Ovis orientalis anatolica</i>	298	298	100	2E-77	99

According to Taxonomy and Genbank NCBI database, the following synonyms refer to mouflon (*Ovis aries musimon* Pallas, 1811): *Ovis orientalis musimon*; *Ovis aries mufflon*; *Ovis musimon*; *Ovis gmelini*; *Ovis ammon musimon*.

Alignment (next page): *Ovis* spp. mtDNA *control region* sequences retrieved from GenBank (BG-1 to 13), with the corresponding GenBank taxonomic identity, and *Ovis* spp. amplicons generated with the primer set HRM_Cer (IRH000213 amplicon from engorged tick collected from a wild sheep; IRQxxxxx amplicons from questing nymphs), together with one *Capreolus capreolus* (IRQ02713) and one *Cervus elaphus* (IRQ039412) amplicons from questing nymphs obtained in this study.

The alignment shows the lack of mutation in the amplified fragment such as to permit the discrimination between *Ovis aries* and its subspecies, justifying the BLASTn search results reported in the previous Table. Additionally, the alignment underline the high intraspecific variability in *Ovis aries* and the lack of defined interspecific mutations compared to *C. capreolus* and *C. elaphus*, explaining the complexity in HRMA amplicons identification with the HRM_Cer primer set.

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GB-1_O.aries_Z35264      CGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACAGTGGTGTGCATGATTTGGTATTTTTTAAT
GB-2_O.aries_Z35258      .....-.....T.....
GB-3_O.aries_musimon_GU350335 .....-.....T.....
GB-4_O.aries_Z35240      .....-.....T.....
GB-5_O.aries_Z35233      .....-.....T.....
GB-6_O.aries_Z35267      .....-.....T.....
GB-7_O.aries_musimon_GU350328 .....-.....T.....
IRQ52513                  .....-.....T.....
IRQ52013                  .....-.....T.....
GB-8_O.aries_musimon_AF039579 .....-.....T.....
GB-9_O.aries_musimon_AY091487 .....-.....T.....
GB-10_O.aries_musimon_HM236184 .....-.....T.....
IRQ15013                  .....-.....T.....
IRH000213                 .....-.....T.....
IRQ071212_A              .....-.....T.....
IRQ059412                 .....-.....T.....
GB-11_O.aries_ophion_KF312238 .....-.....T.....
GB-12_O.aries_Z35249      .....-.....T.....
GB-13_O.aries_AB006801    .....G.....A.....TC.C.....T...C.....A.....
IRQ02713_C.capreolus     .....TC.C.....T.....A.....
IRQ039412_C.elaphus      .....TC.C.....T.....A.....A.....A.T.
Clustal Consensus        ***** ***** * ***** ** ***** ***** ***** *

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GB-1_O.aries_Z35264      TTTTGGGG-ATGCTTGACTCAGCTATGGCCGTCTG-AGGCCCGACCCGGAGCATGAATTGTAGCTGGA
GB-2_O.aries_Z35258      .....-.....T.....
GB-3_O.aries_musimon_GU350335 .....-.....T.....
GB-4_O.aries_Z35240      .....-.....AG.....
GB-5_O.aries_Z35233      .....-.....AG.....
GB-6_O.aries_Z35267      .....-.....AG.....
GB-7_O.aries_musimon_GU350328 .....-.....AG.....
IRQ52513                  .....-.....AG.....
IRQ52013                  .....-.....AG.....
GB-8_O.aries_musimon_AF039579 .....-.....AG.....
GB-9_O.aries_musimon_AY091487 .....-.....AG.....
GB-10_O.aries_musimon_HM236184 .....-.....AG.....
IRQ15013                  .....-.....AG.....
IRH000213                 .....-.....AG.....
IRQ071212_A              .....-.....AG.....
IRQ059412                 .....-.....AG.....
GB-11_O.aries_ophion_KF312238 .....-.....AG.....
GB-12_O.aries_Z35249      .....-.....AG.....
GB-13_O.aries_AB006801    G.C.....A.A.....T.....T.....G
IRQ02713_C.capreolus     .....G.....AA.....A.....
IRQ039412_C.elaphus      ...G...G.....A...A.....G...T...T.....
Clustal Consensus        * **** ***** ** * * * * * * * * * * * * * * * * * * * * * * * *

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GB-1_O.aries_Z35264      CTTAACTGCATCTTGAGCATCCTCATAAT
GB-2_O.aries_Z35258      .....
GB-3_O.aries_musimon_GU350335 .....
GB-4_O.aries_Z35240      .....
GB-5_O.aries_Z35233      .....
GB-6_O.aries_Z35267      .....
GB-7_O.aries_musimon_GU350328 .....C.....
IRQ52513                  .....C...--
IRQ52013                  .....C...--
GB-8_O.aries_musimon_AF039579 .....C.....
GB-9_O.aries_musimon_AY091487 .....C.....
GB-10_O.aries_musimon_HM236184 .....C.....
IRQ15013                  .....C...--
IRH000213                 .....C...--
IRQ071212_A              .....C.....C...--
IRQ059412                 .....C...--
GB-11_O.aries_ophion_KF312238 .....
GB-12_O.aries_Z35249      .....
GB-13_O.aries_AB006801    .....AC.....
IRQ02713_C.capreolus     .....C...--
IRQ039412_C.elaphus      .....C...--
Clustal Consensus        ***** ***** **

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