5. DISCUSSION

In TBD risk evaluation, it is essential to understand which factors are affecting vector population dynamics and the epidemiology of each specific tick-borne disease involved. In fact, in order to estimate the final pathogen transmission potential of the tick itself, it is necessary to consider the effect of both the biotic components of the interactions taking place between the vector, and a diversity of hosts and pathogens; intrinsic biological barriers (i.e. molecular, cellular, physiological and physical); and the abiotic component of the environment in which the TBD system is embedded (Pfäffle et al., 2013; Randolph, 2008; Fig. 5.1). These considerations are particularly important in the present context of rapid variations in climate, socio-demographic factors and land use, which not only strongly impact vector and hosts populations, but also modify the frequency of contacts of humans and domestic animals with infected ticks (which is directly correlated with disease risk).

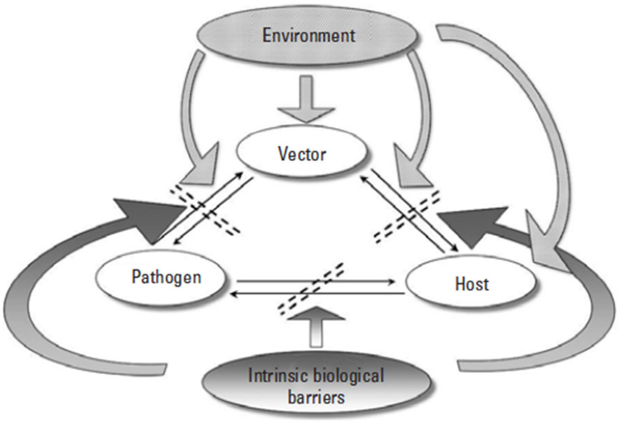

Fig. 5.1 The triangle of host- vector - pathogen interactions, showing the points of action of the intrinsic biological barriers to transmission and the extrinsic environmental factors (reprinted from Randolph et al., 2008).

In this study, I integrated two advanced molecular approaches, NGS population genomics and bloodmeal analysis by HRMA, to infer the host-use pattern of *I. ricinus* at a regional scale and the potential effect of this behavior on TBD epidemiology. First, I will discuss the novelty of the Real-time HRMA bloodmeal analysis protocol we developed and the future technical improvement that would made it more efficient; then, population genomics and bloodmeal analysis results will be discussed in the framework of reference literature.

5.1 A new tool for bloodmeal analysis in questing ticks

Molecular bloodmeal analysis is a milestone in the study of arthropod borne disease epidemiology, as underlined by the increasing body of research on this topic and its application to several hematophagous vectors (tsetse flies, *Anopheles* and *Culex* mosquitoes, reviewed in Kent, 2009; Triatominae bugs, Peña et al., 2012) including ticks (Estrada-Peña et al., 2005; Pichon et al., 2003, 2005, 2006; Humair et al., 2007; Morán Cadenas et al., 2007; Allan et al., 2010; Wodecka et al., 2014). However, although molecular methods can provide high specificity and sensitivity in bloodmeal analysis (Kent, 2009), their resolution is still limited by digestive process and mixed bloodmeals, as is the case of their application in ticks (Gómez-Dìaz and Figuerola, 2010). In fact, while there is urgent need for such a reliable method (Bolzoni et al., 2012; Estrada-Peña et al., 2013), the current molecular methods available for bloodmeal analysis in questing ticks are not considered sufficiently robust for application in field studies (Estrada-Peña et al., 2013), and questions have been raised concerning their susceptibility to contaminations (Collini et al., 2015a in press; Collini et al., 2015b).

Here, I present the novel application of Real-time HRMA as a new tool for the investigation of tick feeding ecology. Before testing the new technique, we explored a variety of DNA sequencing protocols, using universal vertebrate primers as well as species-specific and host group ones, since sequencing would be the most straightforward way to capture the wide host range of the sheep tick and/or to identify hosts to species level. In addition, reduced sequencing costs, as well as improved PCR reagents and instrumentation, were all factors favouring testing the sequencing approach by simply designing new primer sets. Although I did not obtain encouraging results, they provided key indications for the successive and successful design of the Real-time HRMA protocol. That is, the use of universal vertebrate *cytb* primers led to the amplification of contaminant human DNA, so consequently, I went on to design primers that did not cross-react with human DNA. In addition, the superior performance of primers amplifying shorter amplicons moved my primer design toward very short amplicons, and host group primers amplifying species out the range of the selected ones, led me to avoid degenerate primers.

The presented Real-time HRMA protocol, involving the amplification of host DNA by means of six newly designed host group primers, allowed the simultaneous screening and identification of bloodmeal sources in questing ticks for 17 of the most important European vertebrate tick host species (8 wild ungulates, rodents, shrews and birds, as well as the domestic dog, livestock species - goat, sheep and cattle -, and the commensal house mouse), and two genera (*Apodemus* and *Sorex*).

Our results confirmed the power of HRMA to identify the host species or genus from both control DNA samples and questing nymphs. Importantly, HRMA is a non-destructive post-PCR method, meaning that amplification products from questing ticks can be sequenced to confirm species identity in certain cases (e.g. to distinguish *T. philomelos* and *E. rubecula*) and to reach species-level identification for cases in which HRMA can only clarify the genus (e.g. *Sorex* spp. and *Apodemus* spp.). In order to avoid unnecessary expense, I would recommend post-HRMA sequencing for only those amplicons with unusual melting profiles that fall within the T_m range of target species (e.g. sample 12_TR; Appendix-Table A1), or that have similar shape to target species but are outside the reported T_m range. These are worth sequencing as they may extend the T_m range of that species (by identifying intraspecific sequence variation), or even the list of target species for a particular primer set (as in the case reported here of *B. taurus*). However, as shown here, profiles that fall well beyond the range of target species or have a very unusual shape are unlikely to yield host DNA, but are more likely arise from contaminant DNA or primer dimers.

In the first application to questing ticks, we were able to identify bloodmeals in 65.4 % of questing nymphs. This sensitivity is higher than mean sensitivities published thus far for RLBH or RFLP for *I. ricinus* nymphs (24.5 % - 15.5%: Estrada-Peña et al., 2005; 49.4 %: Pichon et al., 2005; 33 %: Pichon et al., 2006; 38.2 %: Humair et al., 2007; 40.6 %: Morán Cadenas et al., 2007) and is similar to that of Wodecka et al. (2014; 62.8 %) and Allan et al. (2010; 62.8%). Our HRMA protocol also proved to have high identification success, correctly assigning host DNA to species or genera using HRMA alone in 40/42 (95.2 %) amplicons obtained from questing nymphs; of these, 35/42 (83.3%) were identified to species. This compares favourably to the 72 % and 62.3 % identification success to genera or species reported in Morán Cadenas et al. (2007) and in Humair et al. (2007), respectively, while all other above-mentioned authors were only able to identify host DNA to group, family or occasionally genus level. Unfortunately, direct comparison of all three currently available methods, by testing the same nymphs with RLBH, RFLP and HRMA, is not possible at this time because of the limited quantity of eluted DNA available from each questing nymph. Only application of this new HRMA protocol to large collections of questing ticks will confirm its place among bloodmeal identification methods.

The lack of host identification in 18 out of 52 questing ticks may be a result of the time since the last bloodmeal, which we estimated as 9 to 14 months (Kirstein and Gray, 1996; Randolph et al., 2002), or specific individual developmental dynamics, heavily affected by site and climatic conditions (Morán Cadenas et al., 2007). However, it may be that some nymphs fed as larvae on species not included in our primer design process (e.g. *Podarcis muralis*, *Erinaceus europaeus*, *L. europaeus*, *S. vulgaris*, *Sus scrofa*, etc.), which are currently considered minor hosts in my study area. For this reason, additional primer sets need to be designed to expand host coverage (see 5.2).

My initial results showed HRMA host misidentification in only one individual, as a result of sequence variation at the intraspecific level, not predictable during amplicon selection and primer design. These errors derive from the relative scarcity of available GenBank mtDNA sequences for some of the selected host-species (e.g. *Apodemus* sp.) that are not currently the object of intensive genetic study, despite their importance in zoonotic disease cycles.

DNA from multiple hosts was detected in 23.5 % of tested nymphs. Even if this result is based on a relatively small number of samples, it is comparable to that obtained in 2007 by Morán Cadenas et al. (19.5 %) and Allan et al. (16.2%; 2010), although the efficacy of PCR-RLBH and PCR-RFLP protocols to detect mixed bloodmeals has not been thoroughly investigated to our knowledge (see Humair et al., 2007; Wodecka et al., 2014). In addition, the presence of DNA from more than one host may increase the complexity of host identification with the above methods as well as those using direct sequencing (Alcaide et al., 2009; Kent, 2009). However, my HRMA approach allows unambiguous detection of multiple hosts, at least when these are species belonging to different host groups. The fact that no nymphs were initially found to have fed as larvae on targeted species of Passeriformes and Caprinae was probably not an indication of the suitability of the primer set, but simply a result of the small sample size; in fact, the bloodmeal screening of the much larger sample using this protocol also resulted in successful amplification of *Passeriformes* and *Caprinae* DNA from questing nymphs.

My results demonstrate that Real-Time HRMA is a reliable method for bloodmeal analysis in questing ticks. Although six different amplifications must be carried out on each tick, the single-step Real-time HRMA design described here, enabling reaction processing, screening and genotyping on the same instrument, still makes it simple and fast compared to other methods. In addition, the method is extremely useful for unambiguous identification of multiple host DNA (mixed bloodmeals). Our protocol also reduces errors common in multi-step molecular protocols and avoids amplification of both environmental and human contaminating DNA, a recurring problem in low quantity/quality DNA studies. Moreover, the lack of the sequencing step in all but the most dubious cases lowers the cost of analysis. In addition, Real-time HRMA reagents have costs comparable to those for conventional PCR, and are usually provided as a *supermix*, further reducing errors (Reed et al., 2007). Although the technique described here is optimized for nymphs, our protocol should also be easily applicable to adult questing ticks, given the larger bloodmeal of the nymphal stage compared to the larval one. This protocol could also be applied to other species of ticks, and other hematophagous insects in general, if this were convenient, by expanding primer sets if necessary.

Use of the Real-time HRMA protocol developed here for the screening of questing nymphs collected from the remaining sampling sites of PAT gave us the possibility to test it on a large sample for a more reliable evaluation of its usefulness and to introduce a more automated configuration. At the same time, important results were obtained regarding the feeding biology of *I. ricinus* larvae in the study area, that would be discussed later.

As time and financial resources are important limiting factors in research, it is important to note that the more automated configuration enabled an increase in time and cost efficiency of the protocol: (i) automated DNA extraction allows the processing of 93 nymphs in one working day by two operators, as well as a two-thirds decrease in reagent costs (magnetic-beads automated Thermo 1.70 € +IVA *vs* manual spin-column Qiagen 4.96 €+IVA); (ii) robotic PCR reaction set-up decreased operator working time and reduced the errors and variability usually related to operator skills; (iii) the RotorGene instrument permitted to process 32 unknown samples at a time.

On the other hand, a notable decrease in identification success (25.4% overall), related to the introduction of the magnetic-beads automatized DNA extraction method, was observed. However, identification success also varied greatly between sampling sites (QIAamp: 31.3 – 92.3%; Thermo: 3.3 – 50.0%). Moràn-Cadenas et al. (2007) also showed that identification success varies according to site-specific climatic conditions (49.9% North facing slope, 41.0 % South facing slope). Since these conditions also direct affect the metabolic rate of ticks and their population dynamics, in the same sampling site, identification success also changed according to sampling time, so that the North facing slope had higher values in spring and autumn (93% May and 73% October) and lower values in summer (20% July), while the South facing slope presented a low identification success from April to July (19 - 35%), with an increase during the summer and the highest peak in October (68%). Even if sampling time did not significantly explain the variation in identification success in our dataset, possibly because samples were all collected in spring, it could be that local micro-climatic variables did have an influence. Unfortunately, we do not have the relevant measurements at our sample sites, such as saturation deficit (the most important climatic variable in regulating tick activity and, therefore, metabolic rate; Tagliapietra et al., 2011), which would have allowed a better investigation of this effect.

As postulated soon after the development of the HRMA protocol, sequencing of amplicons having unusual melting patterns extended the list of target species for Cervidae (*D. dama*, *Ovis* spp. and *R. rupicapra*), Passeriformes (*Turdus* spp.) and Muroidea (*C. griseus*). These additional species are not distantly related to the target species and the mtDNA regions selected show relatively low levels of variability; consequently, in some cases, melting temperatures of these additional species partially overlap with the ones of primary target species, making the use of HRMA for species level identification not as straightforward as initial results suggested. On the other hand, analysis of a much larger dataset still showed that primers do not cross-react with distantly-related species compared to those for which they were designed; hence, sequencing of all amplicons could be avoided if host group or genus identification is considered detailed enough according to the scientific hypothesis of the study or the target of the bloodmeal analysis (i.e. identification of neglected host species; evaluation of a specific species as tick hosts). For example, as our results showed, the classification of host according to Order still gave important insight into larval host distribution in the two major habitat types investigated.

5.2 Real-time HRMA: Technical problems to resolve

The Real-time HRMA protocol presented here gives a reliable indication of the blood feeding habit of ticks in a certain area, as the main host groups and, most importantly, hosts having a central role in the TBD epidemiological cycles in the Alps, are targeted.

Certainly, a higher identification success would help to improve confidence in the results since bias in the species detected (if it exists) could be ruled out. Such success would also give robustness and more credibility to the results of the statistical analyses used to infer hosts relative importance and model host-parasite systems. Manual QiaAMP® DNA Investigator was more efficient in amplifying host DNA, but may not be ideal for a large sample due to its higher costs and time needed to implement it. However, it should be noted that there is now a large variety of magnetic-beads reagent kits available for combined use with the KingFlex®Magnetic Particle Processor, some specifically designed for small samples sizes and forensic applications, such as QiaAMP® DNA Investigator, that could be tested and optimized.

Substantial improvement of the protocol could be made extending its host range coverage and enhancing species level identification by designing new primers for the current target species. Here, an in-deep evaluation of the availability of reference sequences suitable for HRMA from public databases for Reptiles (*Lacerta viridis*, *P. muralis*), other Carnivores (*Meles meles, Martes martes, Mustela nivalis*), and rodents (*Sciurus vulgaris*, *Glis glis*), or other species (i.e. *E. europaeus*, *Sus scrofa*) was done at this time, showing, for some of these species, few sequences data available; genetic resources could be eventually obtained by *de novo* sequencing mtDNA region by means of universal primers. On the other hand, improving species level identification for the currently targeted species could be challenging, as available mtDNA genetic resources were already exhaustively examined to design the primers used here; others were tested and discarded (either by in-vitro by uMelt^{TM} and/or on control samples). However, the species level identification, could be improved, especially for the host group primers for Cervidae (multiple species having partially overlapping T_m), Muroidea (misidentification of *Apodemus* spp. because of intraspecific variation) and Passeriformes (*T. philomelos, Turdus* spp. and *E. rubecula* having overlapping T_m range), by the introduction of unlabeled probes (Reed et al., 2007) or by heteroduplex formation (Cheng et al., 2006).

In future, the development of multiplexed assays (Reed et al., 2007; Seipp et al., 2008; Gori et al., 2012) would further increase the time- and cost-saving properties of the devised method. Moreover, as less total tick DNA would be used in bloodmeal analysis with this method, a comparative analysis of current methods (i.e. RLBH) and the one presented here could be performed. More importantly with more DNA a parallel screening for TBD etiological agents (i.e. *Borrelia* spp., *Rickettsia* spp., *Anaplasma phagocytophylum, Babesia* spp.) could be performed on the same tick, as done in previously published bloodmeal studies (Pichon et al., 2003, 2005, 2006; Morán Cadenas et al., 2007; Allan et al., 2010; Wodecka et al., 2014). As presented in these articles, knowing both the larval host bloodmeal and the pathogens hosted by each tick would permit a more complete investigation of the importance of vertebrate species in tick population maintenance and their role in the epidemiological cycles of TBD, even for those species rarely trapped or not currently considered important in TBD systems. However, it should be kept in mind that simultaneous identification of pathogen and host DNA does not imply reservoir status for the identified species, or vector status for the ticks, as Estrada-Peña et al. (2013) underlined, but additional laboratory studies must be performed before it can be concluded that a particular tick is a vector, or a host is a reservoir of a particular pathogen.

5.3 Tick biology and epidemiological implications

I. ricinus is traditionally depicted as a generalist parasite, able to exploit a wide range of mammals, birds and reptiles (Hoogstraal and Aeschlimann, 1982). The only constraint in host choice appears at the adult stage, when females preferentially feed on large mammals, in order to take the large bloodmeal needed for egg production (Tälleklint and Jaenson, 1994; Mejlon and Jaenson, 1997). In evolutionary terms, it would appear evident that adaptations allowing a parasite to track host responses to infections would make the parasite better equipped to exploit the host than a generalist; on the other hand, generalists might be more efficient at reacting to environmental variation influencing host diversity and availability. Given the intimacy of tick-host physical and physiological interactions taking place during the long bloodmeal (Brossard and Wikel, 2004), one may expect that a high level of specialization would be necessary to overcome the immune barriers of hosts combatting hematophagous parasitism, making exploitation of several host species difficult. Such specialization could lay the foundation for host-race formation, if not speciation, since a barrier to gene flow between tick populations exploiting different hosts could eventually evolve (reviewed in McCoy et al., 2013; Magalhães et al., 2007). Tick ‘races’ exploiting different hosts would also present genetic structuring at different geographical scales, in relation to host vagility, since tick patterns of gene flow and dispersal appear to be largely determined by host movement during infestation (Porretta et al., 2013 and references therein and later shown and explained here). Host-specialization would have profound consequences on the current understanding of tick population dynamics and associated pathogens, since the reaction of each TBD system to climate and land use change will be different according to the host species involved.

Reviewing *I. ricinus* phylogenetics and population genetics literature, McCoy et al. (2013) came to the conclusion that different degrees of host association divergence may exist in local populations of the sheep tick, according to the time of tick population establishment as well as to the stability of the local host community (Fig. 5.2), as observed for other tick species (*I. uriae*, Kempf et al. 2009a; *Rhipicephalus micropilus*, De Meeûs et al., 2010). This conclusion, mainly based on results of microsatellite analysis of *I. ricinus* on a local scale (De Meeûs et al., 2002; Kempf et al., 2009b; Røed et al., 2006; Kempf et al., 2010; Kempf et al., 2011; De Meeûs et al., 2004), could be possibly rejected in light of the inappropriateness of microsatellites as a molecular markers for this species, as revealed by our analysis and highlighted by Quillery et al. (2013). Although the authors mentioned certain anomalies in the STR dataset (mostly null alleles) and recognized their importance in causing part of the heterozygote deficiency observed, instead of sequencing the markers as I did and discovering that their characteristics did not match those of true microsatellites, they suggested that local substructure and sex-biased genetic structure were the most possible biological explanations. Kempf et al. (2011), using the same microsatellites, concluded that host-associated populations seem to occur sympatrically, but in only some of the sites they analyzed. Hence, we can conclude from the literature that no reliable evidence of tick 'races' has yet been published.

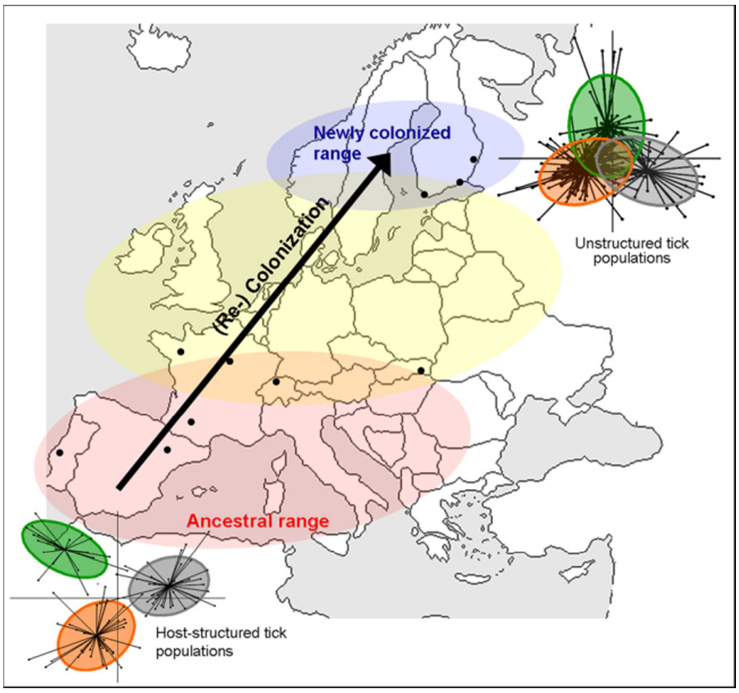

Fig. 5.2 Schematic representation of population expansion of *I. ricinus (arrow)* and hypothesized consequences for host-associated genetic structure. The red zone represents well-established *I. ricinus* populations that persisted in both Southern and Central Europe during the last glacial phase (Porretta et al., 2013). The blue area represents the recently colonized zone for *I. ricinus* and the yellow zone, a transition area where population age and history may be variable (Léger et al., 2013; Porretta et al., 2013). Under the hypothesis that the evolutionary age of a tick population may affect the evolution of host specialization, we would expect a strong pattern of specialization in the red zone, and no host specialization in the blue zone because ticks have only been exposed to local hosts for a few generations. In the yellow zone, patterns of host specialization may be more variable. On the figure, the degree of host-associated population structure of *I. ricinus* in the extreme zones are represented by a between-group analysis of neutral genetic variation, where each dot represents an individual tick and the color indicates different host-associated tick populations. Greater separation of groups indicates stronger genetic divergence. The relative evolutionary age of tick populations could be changed to some other habitat-based factor that may affect the evolution of host specialization. Reprinted from McCoy et al., 2013.

Here I present the first application of RAD-Seq to the study of tick population genetics. The varying sequencing success of individuals may be partially explainable by errors in library preparations; however, similar problems have been observed in other studies using this NGS approach (Xu et al., 2014). The maximum number of RAD markers obtained in this study was significantly smaller than those expected, based on *I. scapularis* genome features (2.3 Gb and 45% GC); as reported in Reitzel et al. (2013 and reference therein), however, it is highly likely that the ratio of expected vs observed RAD-loci was biased by the presence of highly repetitive regions that make up a large fraction of the tick genome (see below) or, as the extremely unsuccessful alignment of *I. ricinus* sequences against the *I. scapularis* genome may suggest, the *I. scapularis* genome parameters used in the RAD-Seq experiment setting may not have been appropriate.

From the extensive analysis of genome wide SNPs retrieved by applying the RAD-Seq protocol on a large number of *I. ricinus* adults collected in the entire distributional range of the species in PAT, and using a variety of bioinformatics approaches, I can conclude that the population of sheep ticks in this area shows no genetic structuring. Individuals had a high degree of admixture and even the two samples from Finland did not cluster separately from the Alpine ones. The Adige River Valley, has previously been identified as a partial barrier to gene flow in red deer, roe deer and chamois populations (Pecchioli et al., 2006; Crestanello et al., 2009), as well as smaller organisms such as mountain hares (Pecchioli et al., 2006), the butterfly, *Erebia euryale* (Haubrich and Schmitt, 2007), and several alpine plants (Schönswetter et al., 2002; Albach et al., 2006). In fact, the area between Lake Garda and Innsbruck, including the Adige Valley, has been invoked as a zone of genetic discontinuity delimiting eastern and western Alpine populations of many other plant species (Thiel-Egenter 2007; Thiel-Egenter et al. 2009). However, the so-called ‘Brenner Line’ did not seem to affect genetic structuring in *I. ricinus* populations.

These findings are in agreement with studies based on mitochondrial (Casati et al., 2008) or on both mitochondrial and nuclear genes (Noureddine et al., 2011; Porretta et al., 2013), showing a lack of phylogeographic structure in the whole range of the mainland European *I. ricinus*, although the European population is clearly divergent from the north-African one (Noureddine et al., 2011). Divergence of the north-African sheep ticks is also supported by microsatellite analysis (De Meeûs et al., 2002) and is likely a consequence of genetic drift following the last Pleistocene glaciation and filling of the Mediterranean sea which now separates the two populations. In fact, seasonal activity and host-association differences have been recorded such that it has been postulated that the introduction of European ticks to Africa by migrating birds would no longer result in reproduction, gene flow and admixture with the native population (Noureddine et al., 2011). Since PAT was completely covered by the Alpine ice-sheet during the Last Glacial Maximum, the absence of geographical genetic structure in the local populations could reflect a rapid expansion of the species after the most recent Pleistocene glaciations, as found at the European level (Noureddine et al., 2011), resulting from a recolonization from the interconnected populations that persisted in both Southern and Central Europe during the Last Glacial Maximum (Porretta et al., 2013), where they presumably parasitized the mammal populations also found in these ‘refugia’ (Taberlet et al., 1998; Hewitt, 1999, 2000; Yannic et al., 2012 and references within). According to McCoy et al. (2013; Fig. 5.2), host-associated divergence is therefore unlikely. However, to explore this hypothesis, the genomic data generated here will be used by another PhD student, Alex Panziera (Univ. of Ferrara), to explore the demographic history of PAT tick populations after he develops software for the analysis of RAD-Seq data.

My results suggest that tick ‘races’ in this area are unlikely, in accordance with McCoy et al. (2013) that suggest a lack of host-association evolution in recently stabilized tick populations. In any case, it appears that the advantages of such specialization are outweighed by positive selection for plasticity for the exploitation of a wide range of hosts. This generalist behavior has had a fundamental role in the persistence of Pleistocene tick populations in suitable areas and in maintaining the interconnections between these populations at a level for which no sign of genetic divergence could be detected (Porretta et al., 2014), as well as in the admixture of present day tick populations (Noureddine et al., 2011; Casati et al., 2008). It will also allow ticks to continue to invade into new regions at higher altitude and latitude (Léger et al., 2013), and peri-urban and urban forest areas (Rizzoli et al., 2014), as a result of climate and land use changes.

In particular, by investigating the genetics of the adult stage, we are looking at the final result of the host-mediated dispersal that acted at the larval and nymphal stages. My results from feeding tick collection and bloodmeal analysis at a local scale both show that even the larval stage may exploit highly vagile wild species, like birds and deer, but also sheep, foxes and dogs. Finally, as suggested in other studies and species, the high presence of repetitive elements and transposable elements that characterize the large tick genomes (Geraci et al., 2007; Nene et al., 2009; Meyer et al., 2010), may provide the genetic variability generating the ability of ticks to exploit such a variety of different hosts (Mastretta-Yanes et al., 2014; Casacuberta and Gonzales, 2013; Sunter et al., 2008). However, in depth study on this topic has not yet been carried out, also because a definitive assembled genome is still lacking, paradoxically, for the very presence of these confounding repetitive elements themselves.

Bird migrations could also account for the recent large scale homogenization of sheep tick populations across mainland Europe, as several studies showed passerines infestation by ixodid ticks also during migration (Humair et al., 1993; Hasle et al., 2011; Comstedt et al., 2006; Waldenström et al., 2007; Lommano et al., 2014; Dubska et al., 2009). Dinnis et al. (2014), founding genetic discontinuity between tick populations in British Isles and Latvia, by using a multilocus sequences typing on mtDNA genes, discussed the effectivity of bird-mediated tick dispersal. As emerges from Figure 5.3 and Fig. 5.4, the British Isles are not part of the main south-west/north-east passerine migrations, which reduces the possibility of incoming birds carrying ticks from north-eastern Europe where Latvia lies. Italy, instead, is clearly part of the north-east, south-west post-breeding migration route and is recognized as a wintering locality for the north-central European breeding population of passerines (Busse, 2001; Spina and Volponi, 2008), and a breeding site for north-African wintering species. The genetic discontinuity found between the mainland and the British Isles, supports the hypothesis that birds play an important role in tick dispersals and could largely explain the lack of geographical structuring in *I. ricinus* ticks in mainland Europe, as well as in PAT, which also lies along the important migration routes described above. Climate change (i.e. increases in the mean temperature), inducing an extended questing period and reducing winter mortality, may additionally enhance tick dispersal by birds during the autumn migrations by increasing the tick-load per bird and also by promoting their survival in the new environment.

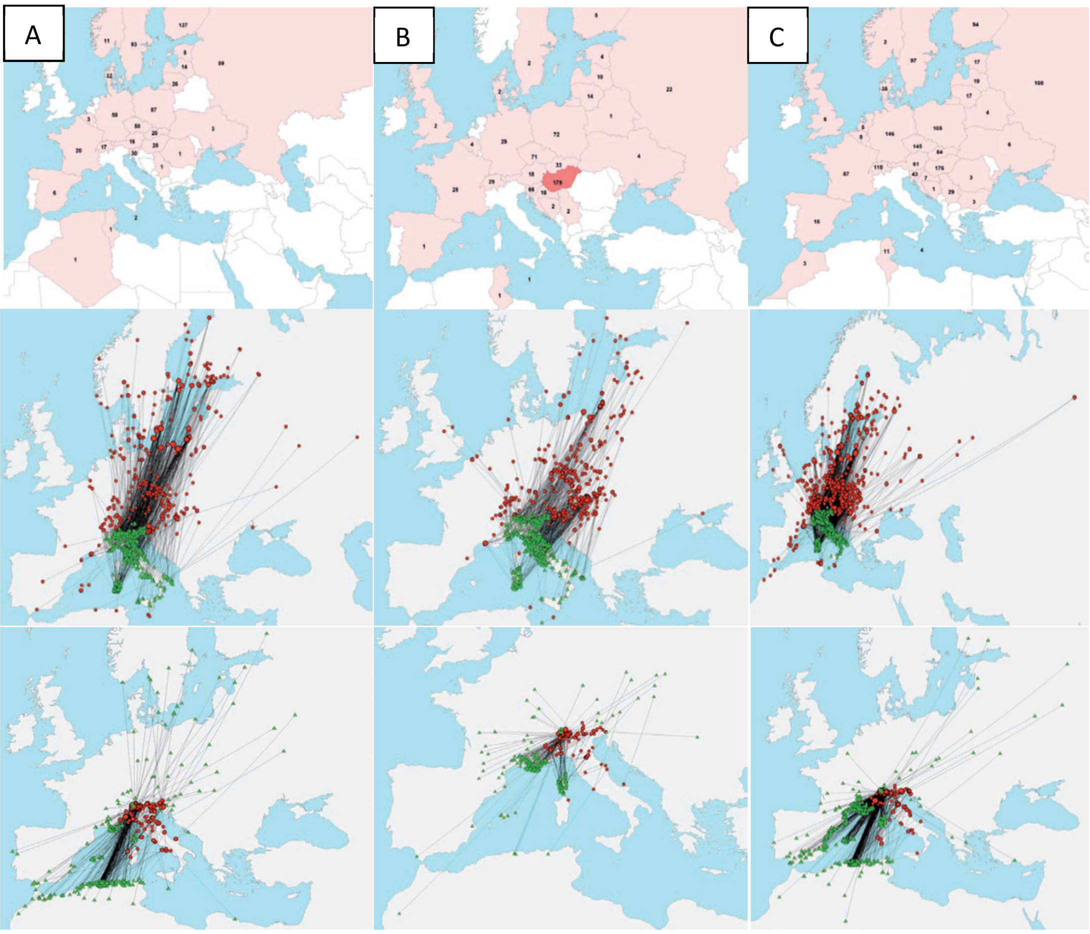

Fig. 5.3 Numbers of foreign ringed birds recovered in Italy for each Country of origin (above); movements of foreign ringed birds recovered in Italy (center), and Italian ringed birds recovered abroad (below). A. *E. rubecula*; B. *T. merula*; C. *T. philomelos* (Spina & Volponi, 2008; ISPRA)

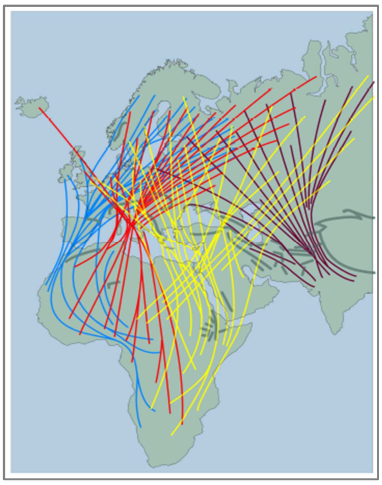

Fig. 5.4 Bird migratory routes at the Eurasian-Africa scale clearly showing Italy at the center of a south-west/north-east route (red), while the British Isles, seem to be part of a more western migration route (blue). Downloaded from: [http://www.seen-net.eu/index.php?pg=mapflyways.](http://www.seen-net.eu/index.php?pg=mapflyways)

Deer (*C. elaphus* and *C. capreolus*) and foxes (*V. vulpes*) play an important role in regional scale admixture of tick population, because of the relevant number of all tick stages they can host and spread with their movement (Vor et al., 2010; Carpi et al., 2008; Dumitrache et al., 2014; Meyer-Kaiser et al., 2012). In PAT, it is well-documented that populations of these species are in a period of consistent growth and establishment in peri-urban and urban forest areas, where they can introduce and maintain tick populations, increasing concern for public health protection (Rizzoli et al., 2014; Mackenstedt et al., 2015). Specifically, adult roe deer (*C. capreolus)* show territorial behavior during spring and summer, but the habit of the dominant male to chase away subadults, could lead to a quick dispersal of parasitizing ticks over long distances. In additional, autumn migrations could cover variable distances, from the usual few thousand metres up to 100 km (Vor et al., 2010; Cagnacci et al., 2011). Unstable and size-variable home ranges and floating individuals often occur in red fox populations making them effective in ticks dispersal (Kolb, 1984; Cavallini, 1996).

In the Alps, including PAT, cattle and sheep (both recognized sheep tick hosts; Gray, 1998; also proven by this study) transhumance has been practiced for centuries; although recently much reduced, this practice still occurs. Cattle transhumance involves a vertical movement of the animals from the valley bottom up to the mountain pastures, while sheep flocks follow longer routes, from the Po Plain up to Alpine summer grazing (Bunce et al., 2004); in both cases, the influence on *I. ricinus* dispersal is almost certainly relevant, allowing ticks to cross geographical barriers rarely attempted by wild ungulates (i.e. large rivers; high mountain chains). The modern introduction of acaricide treatments will have reduced the role of domesticated animals in tick dispersal; however, recent studies have shown an upsurge of cattle pathogens vectored by *I.*

ricinus in wild populations of ungulates and in questing ticks, suggesting, on one hand, the continuing importance of cattle and sheep in tick and related diseases dispersal, and on the other, the presence of neglected wild reservoirs for domesticated animal pathogens ticks (Lòpez-Olvera et al., 2009; Rizzoli et al., 2014 and reference therein). At a global scale, the role of increased worldwide transportation of livestock in new tick species and tick-borne disease introductions in new areas is well-known (reviewed in Léger et al., 2013).

Human-mediated tick dispersal at a local scale can also be attributed to leisure activity accompanied by dogs (i.e. hiking and hunting). The importance of dogs in tick dispersal and tick population maintenance, supported for the first time by our bloodmeal analysis, is related to the potential ability of these animals to introduce infected ticks into the urban environment where a variety of urban-adapted hosts could maintain tick populations (such as foxes) and the epidemiological cycle of the consequently introduced pathogen (passerines birds, rodents and insectivores; Rizzoli et al., 2014).

From the findings of this study the complexity of the TBD system clearly emerges, even at the regional scale, and shows that *I. ricinus* ticks exploit a large variety of competent and non-competent vertebrate hosts, which then disperse the ticks over a wide range of distances according to the vagility of the host species itself. Therefore, the relationship between host biodiversity and zoonotic diseases need to be carefully evaluated according to the diseases and local vertebrate community of interest, in order to give better estimates of TBD prevalence and the contact rate for human populations (reviewed in Pfäffle et al., 2013; Bolzoni et al., 2012). Bloodmeal analysis in questing ticks represents an efficient way to perform such an investigation, as it gives estimates of the actual host use, including the association with vagile hosts that may not always be resident in a certain area (i.e. field observations will not be effective), but that contribute to tick population persistence (maintenance hosts) or to specific TBD introductions and prevalence (reservoir/competent hosts).

The results obtained by the application of the newly developed protocol for bloodmeal analysis in PAT questing ticks, are an interesting example of the importance of this indirect way to monitor tick-host use and the possible epidemiological implications. In fact, our results from PAT were particularly interesting in the light of the recent review of Rizzoli et al. (2014) regarding TBD hazard in peri-urban and urban habitat. Large forest covers over the 50% of the Province and fauna includes most of the species recognized both as important competent reservoirs for *Borrelia burgdorferi* s.l., TBE virus and *Anaplasma phagocytophila,* and as tick maintenance hosts. Villages, as well as the main cities in PAT, are embedded in rural ecosystems where wild hosts could interact with domesticated animals and where employment (rangers, lumberjacks, farmers) and leisure activities (hunting, walking, fishing) may enhance the human contact rate with infected ticks. Therefore, it is epidemiologically relevant that rodents, the most important reservoir host group for both *B. burgdorferi* s.l. and TBEv, and deer, implicated in the tick population maintenance and amplification, are widely exploited as larval bloodmeal source in both EXTF and PATF habitats in PAT (31.7% and 24.5% respectively), establishing the basis for disease hazard as a result of infected tick presence in both habitats (Bolzoni et al., 2012). In PATF sites the presence of passerine ground-foraging birds is more pronounced; the role of this host group in the epidemiology of *B. burgdorferi* s.l. and in feeding TBEv infected ticks is widely recognized (Waldenström et al., 2007; Lommano et al., 2014; Humair et al., 1993; Dubska et al., 2009). Birds and deer may benefit from the presence of accessible field crops and ornamental vegetation for their foraging and sheltering in the PATF habitat, while Soricomorpha, generally negatively affected by habitat fragmentation and agricultural activities (Canova and Fasola, 1991; Spinozzi et al., 2012), are more represented in EXTF. The percentage of bloodmeals obtained from dogs is particularly concerning, and is especially high in the forest patches near urban areas (24.5%). The role of dogs in the epidemiology of TBD is still not defined. They do not appear to be an amplification host for TBEv (Pfeffer and Dobler, 2011), or reservoir hosts for Lyme disease *Borrelia* spp. agents (Bhide et al., 2004); however, they can be heavily infested by ticks and thus, contribute to tick population maintenance. More importantly for human tick bite risk, they could also vector infected ticks, acquired during visits to natural forest, into peri-urban and urban parks and gardens where they may later drop off and parasitize humans (Trotta et al., 2012; Farkas et al., 2014; Rizzoli et al., 2014) or more competent hosts (i.e. passerine birds and rodents).

In PAT, as already pointed out, livestock transhumance is still practiced regularly. Interestingly, wild sheep or mouflon (*O. aries musimom*) populations are also present, often in the same grazing areas, and their distribution partially overlaps with tick current and potential habitat. Unfortunately, we are not currently able to discriminate between bloodmeals derived from wild (*O. aries musimon*) or the domestic sheep (*O. aries*), but it would particularly interesting to do so since it has recently been reported that mouflon could serve as wild reservoir host of *Anaplasma spp.*, as do deer, or other pathogens (various strains of *Babesia* spp.) from domesticated animals (Lòpez-Olvera et al., 2009; Rizzoli et al., 2014 and reference therein). Future research should aim at identifying a more suitable mtDNA molecular marker(s) for this purpose, even if other authors claims this may not be possible, because of their close genetic relatedness (Lorenzini et al., 2011).

The occurrence of multiple bloodmeals in 10.7% of the nymphs screened was observed in previous bloodmeal studies (Morán Cadenas et al., 2007; Allan et al., 2010; Gray et al., 1999); moreover, Gray et al. (1999) observed the occasional collection of semi-engorged larvae by blanket dragging, thus supporting the reliability of mixed bloodmeals findings by molecular analysis in nymphs. The ability to have multiple bloodmeals, and its causes, deserves an in depth analysis. In fact, co-infections in questing ticks at both nymphal and adult stage, has been widely observed (Leutenegger et al., 1999; Kurtenbach et al., 2001; Pichon et., 2003, 2005, 2006; Swanson et al., 2006; Reis et al., 2011), as well as human cases deriving from multiple infection from a single tick bites proved to occur and the derived clinical pathology showed complex patterns that make diagnosis and prophylaxis challenging (Swanson et al., 2006; Mitchell et al., 1996; Nadelman et al., 1997). Multiple infections were explained up to now by transtadial/transovarial pathogen transmission and acquisition of additional pathogens by successive meals taken at different stages (Reis et al., 2011; Swanson et al., 2006); however a significant source of such co-infection could derive from repetitive bloodmeals at the same stage, resulting in the mixed bloodmeal identifications reported here, and elsewhere.

5.4 Future research

The applied methodologies developed and described here provide new knowledge regarding tick-host interactions and provide the basis for improved epidemiological models. The complex interactions taking place in TBD systems and the global changes we are continuously facing stimulate new questions for further research. Specifically for the Real-time HRMA bloodmeal analysis protocol presented here, in addition to the technical improvements presented in section 5.2, further investigations are needed to determine whether mixed bloodmeals of species within the same host group are being overlooked (Albonico et al., 2013; McCarthy et al., 2013). As noted by Morán Cadenas et al. (2007) and in the discussion above, further testing should be also done to confirm whether the multiple host DNA is a result of voluntary drop off and secondary questing by the tick (true mixed bloodmeals), involuntary interrupted feeding, or unsuccessful attachment. It would be interesting to test the improved method with a large sample in urban parks tick populations to validate the growing public health concern that dogs play a significant role in increasing tick-human contact rate, and identify possible control strategies to decrease disease risk.

Analysis of the large genomic data produced for *I. ricinus* by RAD-Seq technology are still far from being concluded. For example, past demographic scenarios are currently being analysed as part of another PhD student at the University of Ferrara. As already pointed out, possible bias in the sequencing results may be related to the high presence of repetitive elements in the genome and/or, according to Van Zee et al. (2013), to the high density of SNPs along the genome that could have promoted allele drop-out because of mutation in the restriction site sequence (Gautier et al., 2012), as also pointed out by Quillery et al. (2013). The availability of a reference genome for *I. ricinus* would largely improve the confidence in SNP calling and the investigation of such genomic features that, as discussed above, may provide the genetic variation necessary to allow ticks to successfully interact with such a large variety of vertebrate species. Identification of SNPs in gene coding regions could be performed thanks to the paired-end RAD-Seq format chosen, which allows the creation of contig sequences, and would permit allocation in a annotated genome and primer design; however, the effectiveness of alignment against annotated genomes of other arthropod species needs to be tested, as alignment against the *I. scapularis* genome has already proven to be unsatisfactory. And, least but not last, the identification of suitable markers for distinguishing male and female ticks, would be very useful for identifying sexes at all life cycle stages, useful for many epidemiological questions, such as sex-biased dispersal and assortative pairing in *I. ricinus*, as reported for microsatellites analysis (De Meeûs et al., 2002; Kempf et al., 2009b; Kempf et al., 2010).

6. CONCLUSIONS

Two molecular methods were optimized here for the first time for *I. ricinus*: bloodmeal analysis in questing nymphs by Real-time HRMA and RAD-Seq NGS for population genetic investigations. These two molecular methods represent innovations in the laboratory practice and analysis of the investigated vector and the results obtained thus far are proof-of-principle of their validity. However, there is still need of technical improvements for the bloodmeal method, and the analysis of the large set of generated RAD-Seq genomic data would benefit from the release of a *I. ricinus* reference genome.

My results from both approaches imply that *I. ricinus* is a medium- to long-distance disperser, mediated by the habit to feed on birds, deer, foxes, dogs and livestock, and it is confirmed by the fact that populations are genetically highly admixed. I also showed for the first time that domestic dogs are important tick hosts in some areas.

This knowledge could be combined with the other biotic and abiotic factors to model TBD incidence and emergence, as well the spread of the vector in new climatic suitable areas. The new bloodmeal analysis protocol could also be applied in other areas to identify important host communities and predict and control TBD dynamics; in fact, I have already had several requests in this regard. The epidemiological relevance of dogs as principal *I. ricinus* larval hosts deserves further specific attention in relation human tick bite risk and to the increasing reported cases of urban infected tick populations.

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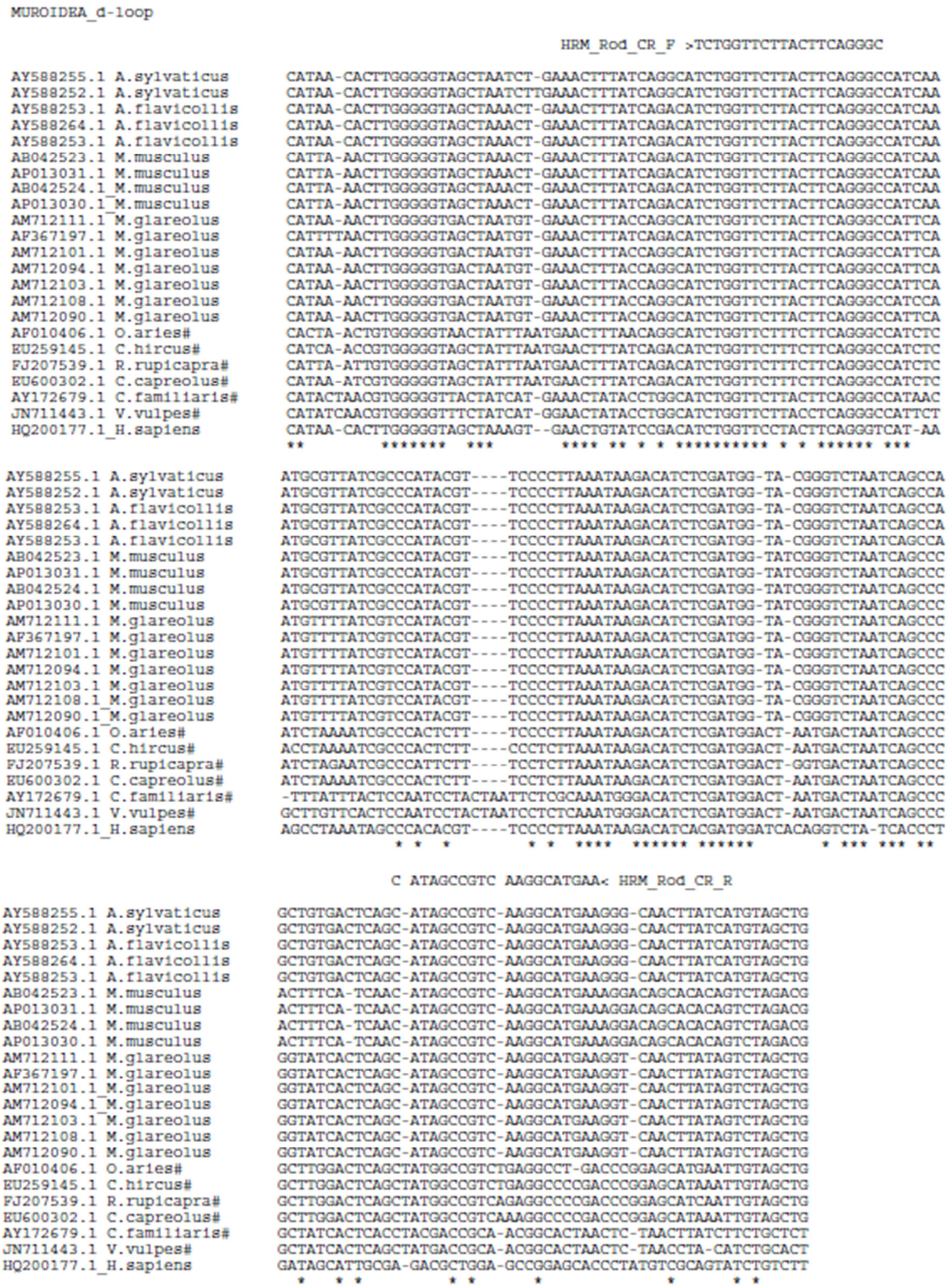
8. APPENDIX

Appendix 1 Part of the host group alignments of previously published GenBank mtDNA sequences created using Clustal X v. 2.0. Details regarding primer positioning.



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MUROIDEA_d-loop

                                                       HRM_Rod_CR_F >TCTGGTTCTTACTTCAGGGC

AY588255.1 A.sylvaticus   CATAA-CACTTGGGGGTAGCTAATCT-GAAACTTTATCAGGCATCTGGTTCTTACTTCAGGGCCATCAA
AY588252.1 A.sylvaticus   CATAA-CACTTGGGGGTAGCTAATCTTGAAACTTTATCAGGCATCTGGTTCTTACTTCAGGGCCATCAA
AY588253.1 A.flavicollis  CATAA-CACTTGGGGGTAGCTAAACT-GAAACTTTATCAGACATCTGGTTCTTACTTCAGGGCCATCAA
AY588264.1 A.flavicollis  CATAA-CACTTGGGGGTAGCTAAACT-GAAACTTTATCAGACATCTGGTTCTTACTTCAGGGCCATCAA
AY588253.1 A.flavicollis  CATAA-CACTTGGGGGTAGCTAAACT-GAAACTTTATCAGACATCTGGTTCTTACTTCAGGGCCATCAA
AB042523.1 M.musculus     CATTA-AACTTGGGGGTAGCTAAACT-GAAACTTTATCAGACATCTGGTTCTTACTTCAGGGCCATCAA
AP013031.1 M.musculus     CATTA-AACTTGGGGGTAGCTAAACT-GAAACTTTATCAGACATCTGGTTCTTACTTCAGGGCCATCAA
AB042524.1_M.musculus     CATTA-AACTTGGGGGTAGCTAAACT-GAAACTTTATCAGACATCTGGTTCTTACTTCAGGGCCATCAA
AP013030.1_M.musculus     CATTA-AACTTGGGGGTAGCTAAACT-GAAACTTTATCAGACATCTGGTTCTTACTTCAGGGCCATCAA
AM712111.1_M.glareolus    CATAA-AACTTGGGGGTGACTAATGT-GAAACTTTACCAGGCATCTGGTTCTTACTTCAGGGCCATTCA
AF367197.1 M.glareolus    CATTTTAACTTGGGGGTAGCTAATGT-GAAACTTTATCAGACATCTGGTTCTTACTTCAGGGCCATTCA
AM712101.1 M.glareolus    CATAA-AACTTGGGGGTGACTAATGT-GAAACTTTACCAGGCATCTGGTTCTTACTTCAGGGCCATTCA
AM712094.1 M.glareolus    CATAA-AACTTGGGGGTGACTAATGT-GAAACTTTACCAGGCATCTGGTTCTTACTTCAGGGCCATTCA
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AM712108.1 M.glareolus    CATAA-AACTTGGGGGTGACTAATGT-GAAACTTTACCAGGCATCTGGTTCTTACTTCAGGGCCATCCA
AM712090.1 M.glareolus    CATAA-AACTTGGGGGTGACTAATGT-GAAACTTTACCAGGCATCTGGTTCTTACTTCAGGGCCATTCA
AF010406.1 O.aries#       CACTA-ACTGTGGGGGTAACTATTTAATGAACTTTAACAGGCATCTGGTTCTTTCTTCAGGGCCATCTC
EU259145.1 C.hircus#      CATCA-ACCGTGGGGGTAGCTATTTAATGAACTTTATCAGACATCTGGTTCTTTCTTCAGGGCCATCTC
FJ207539.1 R.rupicapra#   CATTA-ATTGTGGGGGTAGCTATTTAATGAACTTTATCAGACATCTGGTTCTTTCTTCAGGGCCATCTC
EU600302.1 C.capreolus#   CATAA-ATCGTGGGGGTAGCTATTTAATGAACTTTATCAGACATCTGGTTCTTTCTTCAGGGCCATCTC
AY172679.1 C.familiaris#  CATACTAACGTGGGGGTTACTATCAT-GAAACTATACCTGGCATCTGGTTCTTACTTCAGGGCCATAAC
JN711443.1 V.vulpes#      CATATCAACGTGGGGGTTTCTATCAT-GGAACTATACCTGGCATCTGGTTCTTACCTCAGGGCCATTCT
HQ200177.1_H.sapiens      CATAA-CACTTGGGGGTAGCTAAAGT--GAACTGTATCCGACATCTGGTTCCTACTTCAGGGTCAT-AA
                          **        *******  ***       **** ** * * ********** * * ****** ***

AY588255.1 A.sylvaticus   ATGCGTTATCGCCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TA-CGGGTCTAATCAGCCA
AY588252.1 A.sylvaticus   ATGCGTTATCGCCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TA-CGGGTCTAATCAGCCA
AY588253.1 A.flavicollis  ATGCGTTATCGCCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TA-CGGGTCTAATCAGCCA
AY588264.1 A.flavicollis  ATGCGTTATCGCCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TA-CGGGTCTAATCAGCCA
AY588253.1 A.flavicollis  ATGCGTTATCGCCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TA-CGGGTCTAATCAGCCA
AB042523.1 M.musculus     ATGCGTTATCGCCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TATCGGGTCTAATCAGCCC
AP013031.1 M.musculus     ATGCGTTATCGCCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TATCGGGTCTAATCAGCCC
AB042524.1 M.musculus     ATGCGTTATCGCCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TATCGGGTCTAATCAGCCC
AP013030.1 M.musculus     ATGCGTTATCGCCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TATCGGGTCTAATCAGCCC
AM712111.1 M.glareolus    ATGTTTTATCGTCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TA-CGGGTCTAATCAGCCC
AF367197.1 M.glareolus    ATGTTTTATCGTCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TA-CGGGTCTAATCAGCCC
AM712101.1 M.glareolus    ATGTTTTATCGTCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TA-CGGGTCTAATCAGCCC
AM712094.1 M.glareolus    ATGTTTTATCGTCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TA-CGGGTCTAATCAGCCC
AM712103.1 M.glareolus    ATGTTTTATCGTCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TA-CGGGTCTAATCAGCCC
AM712108.1_M.glareolus    ATGTTTTATCGTCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TA-CGGGTCTAATCAGCCC
AM712090.1_M.glareolus    ATGTTTTATCGTCCATACGT----TCCCCTTAAATAAGACATCTCGATGG-TA-CGGGTCTAATCAGCCC
AF010406.1_O.aries#       ATCTAAAATCGCCCACTCTT----TCCCCTTAAATAAGACATCTCGATGGACT-AATGACTAATCAGCCC
EU259145.1 C.hircus#      ACCTAAAATCGCCCACTCTT----CCCTCTTAAATAAGACATCTCGATGGACT-AATGACTAATCAGCCC
FJ207539.1 R.rupicapra#   ATCTAGAATCGCCCATTCTT----TCCTCTTAAATAAGACATCTCGATGGACT-GGTGACTAATCAGCCC
EU600302.1 C.capreolus#   ATCTAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACT-AATGACTAATCAGCCC
AY172679.1 C.familiaris#  -TTTATTTACTCCAATCCTACTAATTCTCGCAAATGGGACATCTCGATGGACT-AATGACTAATCAGCCC
JN711443.1 V.vulpes#      GCTTGTTCACTCCAATCCTACTAATCCTCTCAAATGGGACATCTCGATGGACT-AATGACTAATCAGCCC
HQ200177.1_H.sapiens      AGCCTAAATAGCCCACACGT----TCCCCTTAAATAAGACATCACGATGGATCACAGGTCTA-TCACCCT
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                                      C ATAGCCGTC AAGGCATGAA< HRM_Rod_CR_R

AY588255.1 A.sylvaticus   GCTGTGACTCAGC-ATAGCCGTC-AAGGCATGAAGGG-CAACTTATCATGTAGCTG
AY588252.1 A.sylvaticus   GCTGTGACTCAGC-ATAGCCGTC-AAGGCATGAAGGG-CAACTTATCATGTAGCTG
AY588253.1 A.flavicollis  GCTGTGACTCAGC-ATAGCCGTC-AAGGCATGAAGGG-CAACTTATCATGTAGCTG
AY588264.1 A.flavicollis  GCTGTGACTCAGC-ATAGCCGTC-AAGGCATGAAGGG-CAACTTATCATGTAGCTG
AY588253.1 A.flavicollis  GCTGTGACTCAGC-ATAGCCGTC-AAGGCATGAAGGG-CAACTTATCATGTAGCTG
AB042523.1 M.musculus     ACTTTCA-TCAAC-ATAGCCGTC-AAGGCATGAAAGGACAGCACACAGTCTAGACG
AP013031.1 M.musculus     ACTTTCA-TCAAC-ATAGCCGTC-AAGGCATGAAAGGACAGCACACAGTCTAGACG
AB042524.1 M.musculus     ACTTTCA-TCAAC-ATAGCCGTC-AAGGCATGAAAGGACAGCACACAGTCTAGACG
AP013030.1 M.musculus     ACTTTCA-TCAAC-ATAGCCGTC-AAGGCATGAAAGGACAGCACACAGTCTAGACG
AM712111.1 M.glareolus    GGTATCACTCAGC-ATAGCCGTC-AAGGCATGAAGGT-CAACTTATAGTCTAGCTG
AF367197.1 M.glareolus    GGTATCACTCAGC-ATAGCCGTC-AAGGCATGAAGGT-CAACTTATAGTCTAGCTG
AM712101.1_M.glareolus    GGTATCACTCAGC-ATAGCCGTC-AAGGCATGAAGGT-CAACTTATAGTCTAGCTG
AM712094.1_M.glareolus    GGTATCACTCAGC-ATAGCCGTC-AAGGCATGAAGGT-CAACTTATAGTCTAGCTG
AM712103.1_M.glareolus    GGTATCACTCAGC-ATAGCCGTC-AAGGCATGAAGGT-CAACTTATAGTCTAGCTG
AM712108.1 M.glareolus    GGTATCACTCAGC-ATAGCCGTC-AAGGCATGAAGGT-CAACTTATAGTCTAGCTG
AM712090.1 M.glareolus    GGTATCACTCAGC-ATAGCCGTC-AAGGCATGAAGGT-CAACTTATAGTCTAGCTG
AF010406.1 O.aries#       GCTTGGACTCAGCTATGGCCGTCTGAGGCCT-GACCCGGAGCATGAATTGTAGCTG
EU259145.1 C.hircus#      GCTTGGACTCAGCTATGGCCGTCTGAGGCCCCGACCCGGAGCATAAATTGTAGCTG
FJ207539.1 R.rupicapra#   GCTTGGACTCAGCTATGGCCGTCAGAGGCCCCGACCCGGAGCATCAATTGTAGCTG
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AY172679.1 C.familiaris#  GCTATCACTCACCTACGACCGCA-ACGGCACTAACTC-TAACTTATCTTCTGCTCT
JN711443.1 V.vulpes#      GCTATCACTCAGCTATGACCGCA-ACGGCACTAACTC-TAACCTA-CATCTGCACT
HQ200177.1_H.sapiens      GATAGCATTGCGA-GACGCTGGA-GCCGGAGCACCCTATGTCGCAGTATCTGTCTT
                            *   * *         * *      *             *      * *
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SORICIDAE_d-loop

                                         HRM_Inset_DL_F  >TCAGCCCAT   GCCGACACAT

NC 006893.1 C.russula        TAAGACATCTCGATGGAC-TAATGACTAATCAGCCCAT---GCCGACACATAACTGTGGTGTCATACATTTG-G
AY769264.1 C.russula         TAAGACATCTCGATGGAC-TAATGACTAATCAGCCCAT---GCCGACACATAACTGTGGTGTCATACATTTG-G
AY769263.1 C.russula         TAAGACATCTCGATGGAC-TAATGACTAATCAGCCCAT---GCCGACACATAACTGTGGTGTCATACATTTG-G
X90952.1 C.russula           TAAGACATCTCGATGGAC-TAATGACTAATCAGCCCAT---GCCGACACATAACTGTGGTGTCATACATTTG-G
X90951.1 S.araneus           TAAGACATCTCGATGGAC-TAATGACTAATCAGCCCAT---GCTCACACATAACTGTGATTTCTTGCATTTG-G
X78798.1 S.araneus           TAAGACATCTCGATGGAC-TAATGACTAATCAGCCCAT---GCTCACACATAACTGTGATTTCTTGCATTTG-G
EU384669.1 S.antinorii       TAAGACATCTCGATGGAC-TAATGACTAATCAGCCCAT---GCTCACACATAACTGTGGTTTCTTGCATTTG-G
EU384681.1 S.antinorii       TAAGACATCTCGATGGAC-TAATGACTAATCAGCCCAT---GCTCACACATAACTGTGGTTTCTTGCATTTG-G
AY918370.1 C.leucodon        CAAGATAACCTATTGGAT--TTTCATGCGTCAGCCCAT---GCCGACACATAACTGTGGTGTCATGCGGTTG-G
AY918369.1_C.leucodon        CAAGATAACCTATTGGAT--TTTCATGCGTCAGCCCAT---GCCGACACATAACTGTGGTGTCATGCGGTTG-G
AY918371.1_C.leucodon        CAAGATAACCTATTGGAT--TTTCATGCGTCAGCCCAT---GCCGACACATAACTGTGGTGTCATGCGGTTG-G
JF510393.1_S.minutus         TAAGACATCTCGATGGAC-TAATGACTAATCAGCCCAT---GCTCACACATAACTGTGATTTCTTGCATTTG-G
AF010406.1 O.aries#          TAAGACATCTCGATGGAC-TAATGACTAATCAGCCCAT---GCCTA-ACATAACTGTGGTGTCATGCATTTG-G
EU259145.1 C.hircus#         TAAGACATCTCGATGGAC-TAATGACTAATCAGCCCAT---GCTCACACATAACTGTGCTGTCATACATTTG-G
EU887455.1 R rupicapra#      TAAGACATCTCGATGGAC-TGGTGACTAATCAGCCCAT---GCTCACACATAACTGTGCTGTCATACATTTG-G
EU600294.1 C.capreolus#      TAAGACATCTCGATGGAC-TAATGACTAATCAGCCCAT---GCTCACACATAACTGTGGTGTCATACATTTG-G
JQ004399.1 C.elaphus#        TAAGACATCTCGATGGAC-TAATGACTAATCAGCCCAT---GCTCACACATAACTGTGGTGTCATACATTTG-G
AF367197.1 C.glareolus#      TAAGACATCTCGATGGTA-CGG-GTCTAATCAGCCCAT---GCCTA-ACATAACTGTGGTCTCGGGCAGTTG-G
AY588252.1 A.sylvaticus#     TAAGACATCTCGATGGTA-CGG-GTCTAATCAGCCAAG----ACTTC-CATAACTGTGGTCTCGAGCAGTTG-G
AY588264.1 A.flavicollis#    TAAGACATCTCGATGGTA-CGG-GTCTAATCAGCCAAG----ACTTC-CATAACTGTGGTCTCGGGCAGTTG-G
EU740415.1 C.familiaris#     GCGTACACGTGCGTACACGTGCGTACACGTGCGTACACGT-GCGTACACGTGCGTACACGTGCGTACACGTGCG
JN711443.1 V.vulpes#         GTACACACGTACGTACACACGTACACACGTACGTACACAC-GTACACACGTACGTACACACGTACACACGTACG
HQ200177.1_H.sapiens         TAAGACATCACGATGGAT-CACAGGTCTATCACCCTATT--AACCACTCACGGGAGCTCT-CCATGCATTTG-G
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NC 006893.1 C.russula        TATCTTTAATTTTTTGGGGG---GA-----GAGCTTGCTA--TGACTCCGC-TAACAT----TTAATCTCGCCAA
AY769264.1 C.russula         TATCTTTAATTTTTTGGGGG---GA-----GAGCTTGCTA--TGACTCCGC-TAACAT----TTAATCTCGCCAA
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X90952.1 C.russula           TATCTTTAATTTTTTGGGGG---GA-----GAGCTTGCTA--TGACTCCGC-TAACAT----TTAATCTCGCCAA
X90951.1 S.araneus           TATCTAATTTTTTTGGGGGG---GGA----GAGCTTGCTA--TGATCCAGC-TAACAT----TTAATCTCGCCAA
X78798.1 S.araneus           TATCTAATTTTTTTGGGGGG---GGA----GAGCTTGCTA--TGATCCAGC-TAACAT----TTAATCTCGCCAA
EU384669.1 S.antinorii       TATTTAATTTTTTTGGGGGG---GGA----GAGCTTGCTA--TGATCCAGC-TAACAT----TTAATTTCGCCAA
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AY918370.1 C.leucodon        TATCTTTAATTTTTAGGGGG---GA-----GAGCTTGCTA--TGACTCCAC-TAACAT----TTAATTTCACCAA
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AY918371.1 C.leucodon        TATCTTTTATTTTGGGGGGG---GC-----GAGCTTGCTA--TGACTCCGC-TAACAT----TTCATATGGCCGA
JF510393.1 S.minutus         TATCTAATTTTTTTGGGGGG---GGA----GAGCTTGCTA--TGATCC---------------------------
AF010406.1 O.aries#          TATTTTTTAATTTT-TGGGG---ATGCTTGGACTCAGCTA--TGGCCGTC--TGAGGC---CT-GACCCGGAGCA
EU259145.1 C.hircus#         TATTTTTTAATTTT-CGGGG---ATGCTTGGACTCAGCTA--TGGCCGTC--TGAGGC---CCCGACCCGGAGCA
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JQ004399.1_C.elaphus#        TATTTTTAATTTTTGGGGGG---ATGCTTGGACTCAGCAA--TGACCGTCT-GGCGGT---CCCGTCCCGGAGCA
AF367197.1 C.glareolus#      TATTTTTTAATTTT--CGGG---ATGGTATCACTCAGC-A--TAGCCGTC---AAGG---CATGAAGGTCAACTT
AY588252.1 A.sylvaticus#     TATTTTTTTATTTT--TAGG---ATGCTGTGACTCAGC-A--TAGCCGTC---AAGG---CATGAAGGGCAACTT
AY588264.1 A.flavicollis#    TATTTTTTTATTTT--TAGG---ATGCTGTGACTCAGC-A--TAGCCGTC---AAGG---CATGAAGGGCAACTT
EU740415.1 C.familiaris#     TACACGTGCGTACACGTGCGTACACGTGCGTACACGTGCG--TACACGTGC-GTACACGTGCGTACACGTACGTA
JN711443.1 V.vulpes#         TACACA--CGTACACACGTACACACGTACGTACACACGTA--CACACGTAC-GTACAC--------ACGTACGTA
HQ200177.1_H.sapiens         TATTTTCGTCT----GGGGG---GTGT-----GCACGCGA--TAGCATTGCGAGACG----CTGGAGCCGGAGCA
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NC 006893.1 C.russula        TGCAGTTGTAGCTGGGCTT--ATTCTCTAT--GGGGGCCGAAGTAGTTATTACAACCGTA
AY769264.1 C.russula         TGCAGTTGTAGCTGGGCTT--ATTCTCTAT--GGGGGCCGAAGTAGTTATTACAACCGTA
AY769263.1 C.russula         TGCAGTTGTAGCTGGGCTT--ATTCTCTAT--GGGGGCCGAAGTAGTTATTACAACCGTA
X90952.1 C.russula           TGCAGTTGTAGCTGGGCTT--ATTCTCTAT--GGGGGCCGAAGTAGTTATTACAACCGTA
X90951.1 S.araneus           ATCAATTGAAGCTGGGCTT--ATTCTTTAT--GGGGGCCGAAAAGATTATGTTATTATGG
X78798.1 S.araneus           ATCAATTGAAGCTGGGCTT--ATTCTTTAT--GGGGGCCGAAAAGATTATGTTATTATGG
EU384669.1 S.antinorii       ATCAGTTGAAGCTGGGCTT--ATTCTCTAT--GGGGGCCGAAAAGGTGATGCTATCATGA
EU384681.1 S.antinorii       ATCAGTTGAAGCTGGGCTT--ATTCTCTAT--GGGGGCCGAAAAGGTAATGCTATCATGA
AY918370.1 C.leucodon        TACAGTTGTAGCTGGGCTT--ATTCTCTAT--GGGGGCCAAAGTAATTATTCTTAGCGTA
AY918369.1 C.leucodon        TACAGTTGTAGCTGGGCTT--ATTCTCTAT--GGGGGCCAAAGTAATTATTCTTAGCGTA
AY918371.1 C.leucodon        TACAGTTGTAGCTGGGCTT--ATTCTCTAT--GGGGGCCGAAGTAGTTATTATTAGCGTA
JF510393.1_S.minutus         ------------------------------------------------------------
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EU259145.1_C.hircus#         TAA-ATTGTAGCTGGACTT----------------AACTGCA-TCTTGAGCATCCCCATA
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EU600294.1 C.capreolus#      TAA-ATTGTAGCTGGACTT----------------AACTGCA-TCTTGAGCATCCCCATA
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AY588252.1 A.sylvaticus#     AT--CATGTAGCTGGACTT--TAAGTGAAG-GGTCATTAATCCACATAACCAAATCATCG
AY588264.1 A.flavicollis#    AT--CATGTAGCTGGACTT--TAAGTGAAG-GATCATTAATCCACATAACCAAATCATCG
EU740415.1 C.familiaris#     CACGTACGTACACGTGCGTACACGTACGTACACGTGCGTACACGTACGTACACGTACGTA
JN711443.1 V.vulpes#         CAC--ACGTACACACGTACACACGTAC--ACACGT------ACGTACGTACAC--ACGTA
HQ200177.1_H.sapiens         CCC-TATGTCGCAGTATCT--GTCTTTG-ATTCCTGCCTCATCCTATTATTTATCGCAC-
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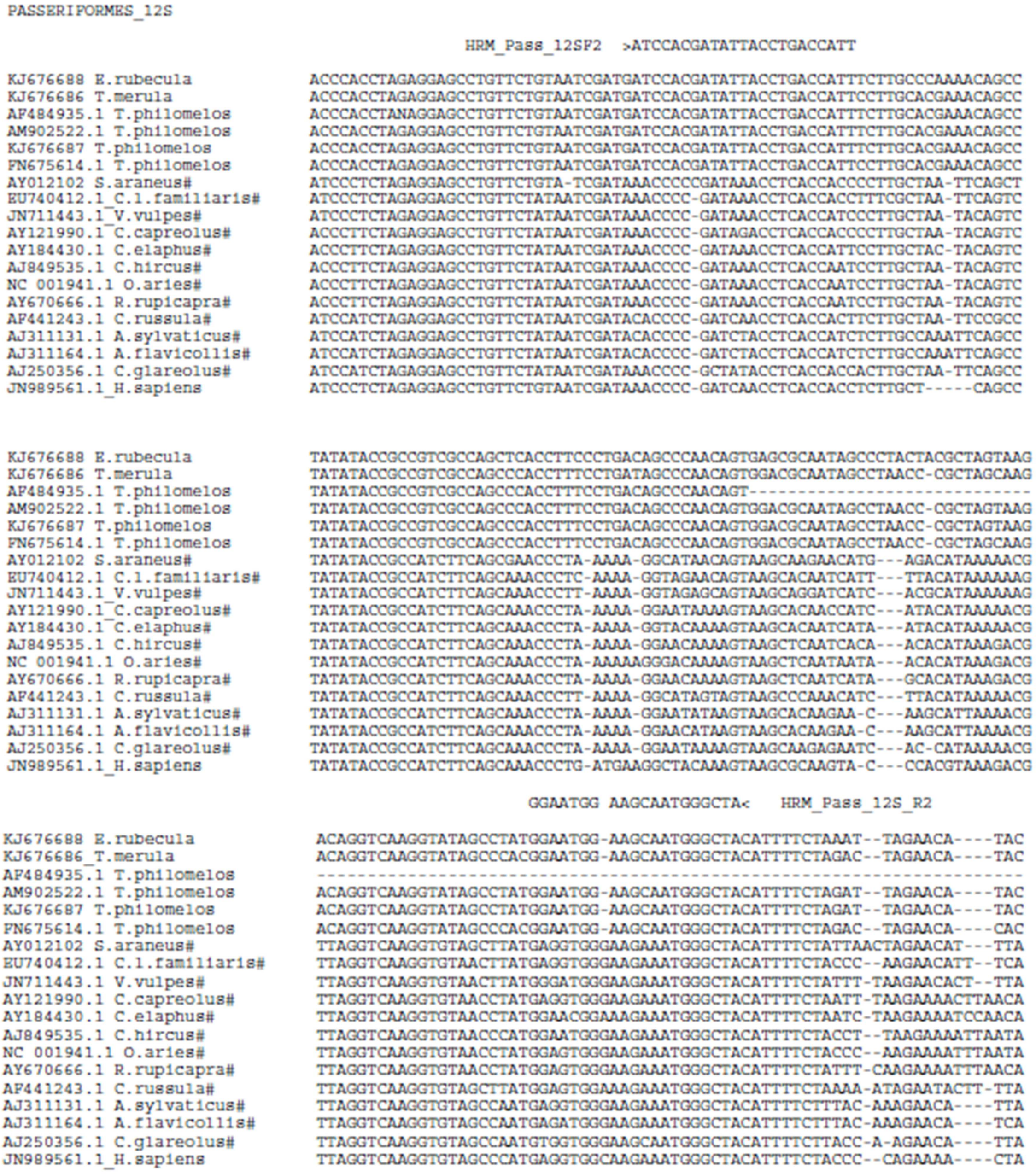

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PASSERIFORMES_12S

                                      HRM_Pass_12SF2  >ATCCACGATATTACCTGACCATT

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KJ676686 T.merula               ACCCACCTAGAGGAGCCTGTTCTGTAATCGATGATCCACGATATTACCTGACCATTCCTTGCACGAAACAGCC
AF484935.1 T.philomelos         ACCCACCTANAGGAGCCTGTTCTGTAATCGATGATCCACGATATTACCTGACCATTTCTTGCACGAAACAGCC
AM902522.1 T.philomelos         ACCCACCTAGAGGAGCCTGTTCTGTAATCGATGATCCACGATATTACCTGACCATTTCTTGCACGAAACAGCC
KJ676687 T.philomelos           ACCCACCTAGAGGAGCCTGTTCTGTAATCGATGATCCACGATATTACCTGACCATTTCTTGCACGAAACAGCC
FN675614.1 T.philomelos         ACCCACCTAGAGGAGCCTGTTCTGTAATCGATGATCCACGATATTACCTGACCATTCCTTGCACGAAACAGCC
AY012102 S.araneus#             ATCCCTCTAGAGGAGCCTGTTCTGTA-TCGATAAACCCCCGATAAACCTCACCACCCCTTGCTAA-TTCAGCT
EU740412.1_C.l.familiaris#      ATCCCTCTAGAGGAGCCTGTTCTATAATCGATAAACCCC-GATAAACCTCACCACCTTTCGCTAA-TTCAGTC
JN711443.1_V.vulpes#            ATCCCTCTAGAGGAGCCTGTTCTATAATCGATAAACCCC-GATAAACCTCACCATCCCTTGCTAA-TACAGTC
AY121990.1_C.capreolus#         ACCCTTCTAGAGGAGCCTGTTCTATAATCGATAAACCCC-GATAGACCTCACCACCCCTTGCTAA-TACAGTC
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AJ849535.1 C.hircus#            ACCCTTCTAGAGGAGCCTGTTCTATAATCGATAAACCCC-GATAAACCTCACCAATCCTTGCTAA-TACAGTC
NC 001941.1 O.aries#            ACCCTTCTAGAGGAGCCTGTTCTATAATCGATAAACCCC-GATAAACCTCACCAATCCTTGCTAA-TACAGTC
AY670666.1 R.rupicapra#         ACCCTTCTAGAGGAGCCTGTTCTATAATCGATAAACCCC-GATAAACCTCACCAATCCTTGCTAA-TACAGTC
AF441243.1 C.russula#           ATCCATCTAGAGGAGCCTGTTCTATAATCGATACACCCC-GATCAACCTCACCACTTCTTGCTAA-TTCCGCC
AJ311131.1 A.sylvaticus#        ATCCATCTAGAGGAGCCTGTTCTATAATCGATACACCCC-GATCTACCTCACCATCTCTTGCCAAATTCAGCC
AJ311164.1 A.flavicollis#       ATCCATCTAGAGGAGCCTGTTCTATAATCGATACACCCC-GATCTACCTCACCATCTCTTGCCAAATTCAGCC
AJ250356.1 C.glareolus#         ATCCATCTAGAGGAGCCTGTTCTATAATCGATAAACCCC-GCTATACCTCACCACCACTTGCTAA-TTCAGCC
JN989561.1_H.sapiens            ATCCCTCTAGAGGAGCCTGTTCTGTAATCGATAAACCCC-GATCAACCTCACCACCTCTTGCT-----CAGCC


KJ676688 E.rubecula             TATATACCGCCGTCGCCAGCTCACCTTCCCTGACAGCCCAACAGTGAGCGCAATAGCCCTACTACGCTAGTAAG
KJ676686 T.merula               TATATACCGCCGTCGCCAGCCCACCTTTCCTGATAGCCCAACAGTGGACGCAATAGCCTAACC-CGCTAGCAAG
AF484935.1 T.philomelos         TATATACCGCCGTCGCCAGCCCACCTTTCCTGACAGCCCAACAGT-----------------------------
AM902522.1 T.philomelos         TATATACCGCCGTCGCCAGCCCACCTTTCCTGACAGCCCAACAGTGGACGCAATAGCCTAACC-CGCTAGTAAG
KJ676687 T.philomelos           TATATACCGCCGTCGCCAGCCCACCTTTCCTGACAGCCCAACAGTGGACGCAATAGCCTAACC-CGCTAGTAAG
FN675614.1 T.philomelos         TATATACCGCCGTCGCCAGCCCACCTTTCCTGACAGCCCAACAGTGGACGCAATAGCCTAACC-CGCTAGCAAG
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AJ250356.1 C.glareolus#         TATATACCGCCATCTTCAGCAAACCCTA-AAAA-GGAATAAAAGTAAGCAAGAGAATC---AC-CATAAAAACG
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                                                    GGAATGG AAGCAATGGGCTA<   HRM_Pass_12S_R2

KJ676688 E.rubecula             ACAGGTCAAGGTATAGCCTATGGAATGG-AAGCAATGGGCTACATTTTCTAAAT--TAGAACA----TAC
KJ676686_T.merula               ACAGGTCAAGGTATAGCCCACGGAATGG-AAGCAATGGGCTACATTTTCTAGAC--TAGAACA----TAC
AF484935.1 T.philomelos         ----------------------------------------------------------------------
AM902522.1 T.philomelos         ACAGGTCAAGGTATAGCCTATGGAATGG-AAGCAATGGGCTACATTTTCTAGAT--TAGAACA----TAC
KJ676687 T.philomelos           ACAGGTCAAGGTATAGCCTATGGAATGG-AAGCAATGGGCTACATTTTCTAGAT--TAGAACA----TAC
FN675614.1 T.philomelos         ACAGGTCAAGGTATAGCCCACGGAATGG-AAGCAATGGGCTACATTTTCTAGAC--TAGAACA----CAC
AY012102 S.araneus#             TTAGGTCAAGGTGTAGCTTATGAGGTGGGAAGAAATGGGCTACATTTTCTATTAACTAGAACAT---TTA
EU740412.1 C.l.familiaris#      TTAGGTCAAGGTGTAACTTATGAGGTGGGAAGAAATGGGCTACATTTTCTACCC--AAGAACATT--TCA
JN711443.1 V.vulpes#            TTAGGTCAAGGTGTAACTTATGGGATGGGAAGAAATGGGCTACATTTTCTATTT-TAAGAACACT--TTA
AY121990.1 C.capreolus#         TTAGGTCAAGGTGTAACCTATGAGGTGGGAAGAAATGGGCTACATTTTCTAATT-TAAGAAAACTTAACA
AY184430.1 C.elaphus#           TTAGGTCAAGGTGTAACCTATGGAACGGAAAGAAATGGGCTACATTTTCTAATC-TAAGAAAATCCAACA
AJ849535.1 C.hircus#            TTAGGTCAAGGTGTAACCCATGGAATGGGAAGAAATGGGCTACATTTTCTACCT--TAAGAAAATTAATA
NC 001941.1 O.aries#            TTAGGTCAAGGTGTAACCTATGGAGTGGGAAGAAATGGGCTACATTTTCTACCC--AAGAAAATTTAATA
AY670666.1 R.rupicapra#         TTAGGTCAAGGTGTAACCTATGGAGTGGGAAGAAATGGGCTACATTTTCTATTT-CAAGAAAATTTAACA
AF441243.1 C.russula#           TTAGGTCAAGGTGTAGCTTATGGAGTGGAAAGAAATGGGCTACATTTTCTAAAA-ATAGAATACTT-TTA
AJ311131.1 A.sylvaticus#        TTAGGTCAAGGTGTAGCCAATGAGGTGGGAAGAAATGGGCTACATTTTCTTTAC-AAAGAACA----TTA
AJ311164.1 A.flavicollis#       TTAGGTCAAGGTGTAGCCAATGAGATGGGAAGAAATGGGCTACATTTTCTTTAC-AAAGAACA----TCA
AJ250356.1 C.glareolus#         TTAGGTCAAGGTGTAGCCAATGTGGTGGGAAGCAATGGGCTACATTTTCTTACC-A-AGAACA----TTA
JN989561.1_H.sapiens            TTAGGTCAAGGTGTAGCCCATGAGGTGGCAAGAAATGGGCTACATTTTCTACCC--CAGAAAA----CTA
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CANIDAE_d-loop

                                     HRM_can_DL_F  >CC       GCA  ACGGCACTAACTC TA

JN711443.1 V.vulpes           ACTTGCTATCACTCAGCTATGACC-------GCA--ACGGCACTAACTC-TAACCTA-CATCTGCACTCAG
GQ374180.1 V.vulpes           ACTTGCTATCACTCAGCTATGACC-------GCA--ACGGCACTAACTC-TAACCTA-CATCTGCACTCAG
AM181037.1 V.vulpes           ACTTGCTATCACTCAGCTATGACC-------GCA--ACGGCACTAACTC-TAACCTA-CATCTGCACTCAG
AY177656.1 C.familiaris       ATCTGCTATCACTCACCTACGACC-------GCA--ACGGCACTAACTC-TAACTTATCTTCTGCTCTCAG
AY177644.1 C.familiaris       ATCTGCTATCACTCACCTACGACC-------GCA--ACGGCACTAACTC-TAACTTATCTTCTGCTCTCAG
AY177652.1 C.familiaris       ATCTGCTATCACTCACCTACGACC-------GCA--ACGGCACTAACTC-TAACTTATCTTCTGCTCTCAG
AY177660.1 C.familiaris       ATCTGCTATCACTCACCTACGACC-------GCA--ACGGCACTAACTC-TAACTTATCTTCTGCTCTCAG
AY172678.1 C.familiaris       ATCTGCTATCACTCACCTACGACC-------GCA--ACGGCACTAACTC-TAACTTATCTTCTGCTCTCAG
AY172679.1 C.familiaris       ATCTGCTATCACTCACCTACGACC-------GCA--ACGGCACTAACTC-TAACTTATCTTCTGCTCTCAG
NC 002008.4 C.familiaris      ATCTGCTATCACTCACCTACGACC-------GCA--ACGGCACTAACTC-TAACTTATCTTCTGCTCTCAG
EU740415.1_C.familiaris       ATCTGCTATCACTCACCTACGACC-------GCA--ACGGCACTAACTC-TAACTTATCTTCTGCTCTCAG
FJ817364.1_C.familiaris       ATCTGCTATCACTCATCTACGACC-------GCA--ACGGCACTAACTC-TAACTTATCTTCTGCTCTCAG
AY656751.1_C.familiaris       ATCTGCTATCACTCACCTACGACC-------GCA--ACGGCACTAACTC-TAACTTATCTTCTGCTCTCAG
AY656749.1 C.familiaris       ATCTGCTATCACTCACCTACGACC-------GCA--ACGGCACTAACTC-TAACTTATCTTCTGCTCTCAG
AY588264.1 A.flavicollis#    --ATGCTGTGACTCAGC-ATAGCC-------GTC--AAGGCAT-GAAGGGCAACTTATCATGTAGCTGGAC
AY588252.1 A.sylvaticus#     --ATGCTGTGACTCAGC-ATAGCC-------GTC--AAGGCAT-GAAGGGCAACTTATCATGTAGCTGGAC
AF367197.1 C.glareolus#      --ATGGTATCACTCAGC-ATAGCC-------GTC--AAGGCAT-GAAGGTCAACTTATAGTCTAGCTGGAC
JQ004399.1 C.elaphus#        --ATGCTTGGACTCAGCAATGACC-------GTCTGGCGGTCCCGTCCCGGAGCATGAATTGTAGCTGGAC
EU600314.1 C.capreolus#      --ATGCTTGGACTCAGCTATGGCC-------GTC-AAAGGCCCCGACCCGGAGCATAAATTGTAGCTGGAC
EU259145.1 C.hircus#         --ATGCTTGGACTCAGCTATGGCC-------GTC-TGAGGCCCCGACCCGGAGCATAAATTGTAGCTGGAC
AF010406.1 O.aries#          --ATGCTTGGACTCAGCTATGGCC-------GTC-TGAGGCCT-GACCCGGAGCATGAATTGTAGCTGGAC
EU887455.1 R rupicapra#      --ATGCTTGGACTCAGCTATGGCC-------GTC-AAAGGCCCCGACCCGGAGCATCAATTGTAGCTGGAC
AY769263.1 C.russula#        GCTTGCTATGACTCCGCTAACATTTAATCTCGCC-AATGCAGTTGTAGCTGGGCTTATTCTCTATGGGGGC
AY918370.1 C.leucodon#       GCTTGCTATGACTCCACTAACATTTAATTTCACC-AATACAGTTGTAGCTGGGCTTATTCTCTATGGGGGC
X78798.1 S.araneus#          GCTTGCTATGATCCAGCTAACATTTAATCTCGCC-AAATCAATTGAAGCTGGGCTTATTCTTTATGGGGGC
HQ200179.1_H.sapiens          ------TATGCACGCGATAGCATT-------GCG-GGAC--GCTGGAGCCGGAGC-ACCCTATGTCGCAGT

JN711443.1 V.vulpes           GGAATATGCCCGTCGCGGCCCCGACGCAGTCAG-ATGATCTGTAGCTGGACTTATTCATTA------TCATT
GQ374180.1 V.vulpes           GGAATATGCCCGTCGCGGCCCCGACGCAGTCAG-ATGATCTGTAGCTGGACTTATTCATTA------TCATT
AM181037.1 V.vulpes           GGAATATGCCCGTCGCGGCCCCGACGCAGTCAG-ATGATCTGTAGCTGGACTTATTCATTA------TCATT
AY177656.1 C.familiaris       GGAATATGCCCGTCGCGGCCCTAACGCAGTCAA-ATAACTTGTAGCTGGACTTATTCATTA------TCATT
AY177644.1 C.familiaris       GGAATATGCCCGTCGCGGCCCTAACGCAGTCAA-ATAACTTGTAGCTGGACTTATTCATTA------TCATT
AY177652.1 C.familiaris       GGAATATGCCCGTCGCGGCCCTAACGCAGTCAA-ATAACTTGTAGCTGGACTTATTCATTA------TCATT
AY177660.1 C.familiaris       GGAATATGCCCGTCGCGGCCCTAATGCAGTCAA-ATAACTTGTAGCTGGACTTATTCATTA------TCATT
AY172678.1 C.familiaris       GGAATATGCCCGTCGCGGCCCTAATGCAGTCAA-ATAACTTGTAGCTGGACTTATTCATTA------TCATT
AY172679.1 C.familiaris       GGAATATGCCCGTCGCGGCCCTAATGCAGTCAA-ATAACTTGTAGCTGGACTTATTCATTA------TCATT
NC 002008.4 C.familiaris      GGAATATGCCCGTCGCGGCCCTAATGCAGTCAA-ATAACTTGTAGCTGGACTTATTCATTA------TCATT
EU740415.1 C.familiaris       GGAATATGCCCGTCGCGGCCCTAATGCAGTCAA-ATAACTTGTAGCTGGACTTATTCATTA------TCATT
FJ817364.1 C.familiaris       GGGATATGCCCGTCGCGGCCCTAATGCAGTCAA-ATAACTTGTAGCTGGACTTATTCATTA------TCATT
AY656751.1_C.familiaris       GGAATATGCCCGTCGCGGCCCTAACGCAGTCAA-ATAACTTGTAGCTGGACTTATTCATTA------TCATT
AY656749.1_C.familiaris       GGAATATGCCCGTCGCGGCCCTAACGCAGTCAA-ATAACTTGTAGCTGGACTTATTCATTA------TCATT
AY588264.1_A.flavicollis#    TTTAAGTGAAGGATCATTAATCCACATAACCAA-ATCATCGA-AGGCTAATATCT---TAA------TGCTT
AY588252.1 A.sylvaticus#     TTTAAGTGAAGGGTCATTAATCCACATAACCAA-ATCATCGC-AGACTAATTAAT---TAA------TGTTT
AF367197.1 C.glareolus#      TTCCTATTAAGTATCATTTATCCCCATCAATAC-CCCTGCAACAGATTAATTAATGGCTCA------GGACA
JQ004399.1 C.elaphus#        TT--AACTGCATCTTGAGCATCCCCATAATGGT-AGGCATGG-GCATG--GCAGT---CAA------TGGT-
EU600314.1 C.capreolus#      TT--AACTGCATCTTGAGCATCCCCATAATGGT-AGGCATGG-ACATG--GCAGT---CAA------TGGT-
EU259145.1 C.hircus#         TT--AACTGCATCTTGAGCATCCCCATAATGGT-AGGCATGG-GCAT--TGCAGT---TAA------TGGT-
AF010406.1 O.aries#          TT--AACTGCATCTTGAGCATCCTCATAATGGT-AAGCATGG-GCATAATATAAT---TAA------TGGT-
EU887455.1 R rupicapra#      TT--AACTGCATCTTGAGCATCCCCATAATGGT-AAGCATGG-GCAT---CGAGT---TAA------TGGT-
AY769263.1 C.russula#        CGAAGTAGTTATTACAACCGTACTTATTCTCTTGATATGGTACATATGGTTTAATGATCTAAAGACATAACT
AY918370.1 C.leucodon#       CAAAGTAATTATTCTTAGCGTACTTATTCTCTTGATAGAGTACATTTAACTTAATGATTTTAAAACATAATG
X78798.1 S.araneus#          CGAAAAGATTATGTTATTATGGCTATTTCTCTT-GCAGGGAGTAAATACGTTTAATGGTTA------CAGGA
HQ200179.1_H.sapiens          --ATCTGTCTTTGAT-TCCTGCCTCATCCTATT-ATTTATCGCACCTACGTTCAATA-TTA------CAGGC

                                                               CAAGGTGCTATTC   AGTCAATGG< HRM_can_DL_R

JN711443.1 V.vulpes           TATCA---ACTCCG---TGCACAAT-------TCAAGGTGCTATTC---AGTCAATGGTTTCAGGACATAA
GQ374180.1 V.vulpes           TATCA---ACTCCG---TGCACAAT-------TCAAGGTGCTATTC---AGTCAATGGTTTCAGGACATAA
AM181037.1 V.vulpes           TATCA---ACTCCG---TGCACAAT-------TCAAGGTGCTATTC---AGTCAATGGTTTCAGGACATAA
AY177656.1 C.familiaris       TATCA---ACTC-A---CGCATAAAA------TCAAGGTGCTATTC---AGTCAATGGTTTCAGGACATAT
AY177644.1 C.familiaris       TATCA---ACTC-A---CGCATAAAA------TCAAGGTGCTATTC---AGTCAATGGTTTCAGGACATAT
AY177652.1 C.familiaris       TATCA---ACTC-A---CGCATAAAA------TCAAGGTGCTATTC---AGTCAATGGTTTCAGGACATAT
AY177660.1 C.familiaris       TATCA---ACTC-A---CGCATAAAA------TCAAGGTGCTATTC---AGTCAATGGTTTCAGGACATAT
AY172678.1 C.familiaris       TATCA---ACTC-A---CGCATAAAA------TCAAGGTGCTATTC---AGTCAATGGTTTCAGGACATAT
AY172679.1 C.familiaris       TATCA---ACTC-A---CGCATAAAA------TCAAGGTGCTATTC---AGTCAATGGTTTCAGGACATAT
NC 002008.4 C.familiaris      TATCA---ACTC-A---CGCATAAAA------TCAAGGTGCTATTC---AGTCAATGGTTTCAGGACATAT
EU740415.1 C.familiaris       TATCA---ACTC-A---CGCATAAAA------TCAAGGTGCTATTC---AGTACATGGT---AGTACACGT
FJ817364.1 C.familiaris       TATCA---ACTC-A---CGCATAAGA------TCAAGGTGCTATTC---AGTCAATGGTTTCAGGACATAT
AY656751.1_C.familiaris       TATCA---ACTC-A---CGCATAAAA------TCAAGGTGCTATTC---AGTCAATGGTTTCAGGACATAT
AY656749.1_C.familiaris       TATCA---ACTC-A---CGCATAAAA------TCAAGGTGCTATTC---AGTCAATGGTTTCAGGACATAT
AY588264.1_A.flavicollis#    TTTCG---GACATA---TTACTTTAA------TTTCACTTGTTTTTCCTAGTTAAAAAATTTTTGACGACA
AY588252.1 A.sylvaticus#     TCTCG---GACATA---TTACTTTAA------TTTCACTTAATTATTATAATTAAAAATTTTATACCAATA
AF367197.1 C.glareolus#      TAACA---CAAGCA---GTACTAATA------ATGCAAATGCTTTTTCCACCTACCAACCC-----CCGTA
JQ004399.1 C.elaphus#        CACAG---GACATA---ATCATTATT------TCATGAGTCAACCCT---AAGATCTATTTCCCCCCCCTG
EU600314.1 C.capreolus#      AGCAG---GACATA---ATTATTATT------TCACGACTCAACCCT---ACTATTCTTTTCCCCCCCCTT
EU259145.1 C.hircus#         CACAG---GACATA---TTTATTATG------TTGCATTTCA---------TCATGCATCCGCTCCACCTT
AF010406.1 O.aries#          CACAG---GACATA---TCTGCTGTA------TCGTGCAT-----------TTATATATTC------TTTT
EU887455.1 R rupicapra#      CACAG---GACATA---ACTATTACG------TCGCACATC----------CCTTATA----------CTT
AY769263.1 C.russula#        TAAGAAGGCTCATAAAGTCTATTGTCAGACTTAATTGTATTTACTTGCTAACATACTATTAAGAGCAATTA
AY918370.1 C.leucodon#       ATTCA---AGAAGA---CTTAGAAATTTATTGTCTGACTTAATTTTATTTACTTGCTAGCATATTATTATA
X78798.1 S.araneus#          CATAAATTGACAAA---ACTGGTGTAAGTACAGGAGAAATGGATTCAG-GTAAAAATAATCAATGGTCACA
HQ200179.1_H.sapiens          GA-------ACATA---CTTACTA------------AAGTGTGTT--------AATTAATTAATGCTTGTA
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CAPRINAE_12S
                                   HRM_Ov1_12S_F >TAAATCTCGTGCCAGCCA

GU350354.1 O.aries           GACTAAGCCATATTGACC--AGGGTTGGTAAATCTCGTGCCAGCCACCGCGGTCATACGATTGACCCAAG
GU350352.1 O.aries           GACTAAGCCATATTGACC--AGGGTTGGTAAATCTCGTGCCAGCCACCGCGGTCATACGATTGACCCAAG
GU229279.1 C.hircus          GACTAAGCCATGTTGACC--AGGGTTGGTAAATCTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAG
HM236185.1 O.aries           GACTAAGCCATATTGACC--AGGGTTGGTAAATCTCGTGCCAGCCACCGCGGTCATACGATTGACCCAAG
HM236179.1 O.aries           GACTAAGCCATATTGACC--AGGGTTGGTAAATCTCGTGCCAGCCACCGCGGTCATACGATTGACCCAAG
HM236184.1 O.aries           GACTAAGCCATATTGACC--AGGGTTGGTAAATCTCGTGCCAGCCACCGCGGTCATACGATTGACCCAAG
NC 001941.1 O.aries          GACTAAGTCATATTGACC--AGGGTTGGTAAATCTCGTGCCAGCCACCGCGGTCATACGATTGACCCAAG
FJ207539.1 R.rupicapra       GACTAAGCCATATTGATT--AGGGTTGGTAAATCTCGTGCCAGCCACCGCGGTCATACGATTAACCCGAG
AY670666.1 R.rupicapra       GACTAAGCCATATTGATT--AGGGTTGGTAAATCTCGTGCCAGCCACCGCGGTCATACGATTAACCCGAG
AM158314.1_R.rupicapra       --------------------------GGTAAATCTCGTGCCAGCCACCGCGGTCATACGATTAACCCGAG
AJ849535.1_C.hircus          --------------------------GGTAAATCTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAG
AY184430.1_C.elaphus         GACTAAGCCATATTAATC--AGGGTTGGTAAATTTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAG
AY184439.1 C.capreolus       GACTAAGCCATATTAATT--AGGGTTGGTAAATCTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAG
AJ311164.1 A.flavicollis     GACTAAGCTATACCTCTA--AGGGTTGGTAAATCTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAA
AJ311131.1 A.sylvaticus      GACTAAGCTATACCTCTA--AGGGTTGGTAAATTTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAA
AJ250356.1 C.glareolus       GACTTAGTCATGCCTCTTC-AGGGTTGGTAAATTTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAA
JN711443.1 V.vulpes          GACTAAGTTATACTAAAG--AGGGTTGGTAAATTTCGTGCCAGCCACCGCGGTCATACGATTAACCCGAA
EU740412.1 C.l.familiaris    GACTAAGCCATACTAAAT--AGGGTTGGTAAATTTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAA
AY012102 S.araneus           GACTAAGTTATGCTAACAT-AGGGTTGGTAAATTTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAA
AF484935.1 T.philomelos      GACTTAGCCATAGCAAATC-AGAGCCGGTAAATCCTGTGCCAGCCACCGCGGTCATACAGGAGGCTCAAA
JN989561.1_Homo              AACTAAGCTATACTAACCCCAGGGTTGGTCAATTTCGTGCCAGCCACCGCGGTCACACGATTAACCCAAG


GU350354.1 O.aries           CTAACAGGAGTACGGCGTAAAGCGTGTTAA--------AGCATCATACTAAATAGAGTTAAATTTTAATTA
GU350352.1 O.aries           CTAACAGGAGTACGGCGTAAAGCGTGTTAA--------AGCATCATACTAAATAGAGTTAAATTTTAATTA
GU229279.1 C.hircus          CTAACAGGAATACGGCGTAAAACGTGTTAA--------AGCACTACATCAAATAGAGTTAAATTCTAATTA
HM236185.1 O.aries           CTAACAGGAGTACGGCGTAAAGCGTGTTAA--------AGCATCATACTAAATAGAGTTAAATTTTAATTA
HM236179.1 O.aries           CTAACAGGAGTACGGCGTAAAGCGTGTTAA--------AGCATCATACTAAATAGAGTTAAATTTTAATTA
HM236184.1 O.aries           CTAACAGGAGTACGGCGTAAAGCGTGTTAA--------AGCATCATACTAAATAGAGTTAAATTTTAATTA
NC 001941.1 O.aries          CTAACAGGAGTACGGCGTAAAGCGTGTTAA--------AGCATCATACTAAATAGAGTTAAATTTTAATTA
FJ207539.1 R.rupicapra       TTAACAGGAATACGGCGTAAAACGTGTTAA--------AGCACCTCACAAAATAGAGTTAAATATTAGTTA
AY670666.1 R.rupicapra       TTAACAGGAATACGGCGTAAAACGTGTTAA--------AGCACCTCACAAAATAGAGTTAAATATTAGTTA
AM158314.1_R.rupicapra       CTAACAGGAATACGGCGTAAAACGTGTTAA--------AGCACCTCACCAAATAGAGTTAAATATTAGTTA
AJ849535.1_C.hircus          CTAACAGGAATACGGCGTAAAACGTGTTAA--------AGCACTACATCAAATAGAGTTAAATTCTAATTA
AY184430.1_C.elaphus         TTAATAGGCACACGGCGTAAAGCGTGTTAA--------AGCACTATACTAAATAAAGTTAAATTCCAATTA
AY184439.1 C.capreolus       TTAATAGGCACACGGCGTAAAGCGTGTTAA--------AGCATTATATAAAATAAGGTTCAATTCTAATTA
AJ311164.1 A.flavicollis     CTAATTACTTCTCGGCGTAAAACGTGCTAAC-------TAGAA-ATTACAAATAGAACTAAAATCCAACTA
AJ311131.1 A.sylvaticus      CTAATTACCTCTCGGCGTAAAACGTGCTAAC-------TAGAA-ACAACAAATAGAACTAAAATCCAACTA
AJ250356.1 C.glareolus       CTAATTA-TTCTCGGCGTAAAACGTGTTACT-------GGGACCACAAAAAATAGAATTGAAATCCATCCA
JN711443.1 V.vulpes          CTAATAGGCCCACGGCGTAAAGCGTGTTTAA-------GATAACATATTAC-TAAAGTTAAAACTTAACTA
EU740412.1 C.l.familiaris    CTAATAGGCCTACGGCGTAAAGCGTGTTCAA-------GATACTTTTACAC-TAAAGTTAAAACTTAACTA
AY012102 S.araneus           TTAATAGGCA-ACGGCGTAAAGCGTGTTAAAGAAAG-CTATACCCACACAAATAAAGACAAAATTTAACTA
AF484935.1 T.philomelos      TTAACTTTATAACGGCGTAAAGAGTGGTCGCA-----TGTTATCCAAGTAGCTAAGATTAAAAAGCAACTG
JN989561.1_Homo              TCAATAGAAGC-CGGCGTAAAGAGTGTTTTAGA----TCACCCCCTCCCCAATAAAGCTAAAACTCACCTG

                                                                  ATGACGAAAGTAACCCTAC<  HRM_Ov1_12S_R

GU350354.1 O.aries           AACTGTAAAAAGCCATAATT--ATAACAAAAAT--AAATGACGAAAGTAACCCTACAATAGC------TGA
GU350352.1 O.aries           AACTGTAAAAAGCCATAATT--ATAACAAAAAT--AAATGACGAAAGTAACCCTACAATAGC------TGA
GU229279.1 C.hircus          AACTGTAAAAAGCCATAATT--ACAACAAAAAT--AGATGACGAAAGTAACCCTACTGCAGC------TGA
HM236185.1 O.aries           AACTGTAAAAAGCCATAATT--ATAACAAAAAT--AAATGACGAAAGTAACCCTACAATAGC------TGA
HM236179.1 O.aries           AACTGTAAAAAGCCATAATT--ATAACAAAAAT--AAATGACGAAAGTAACCCTACAATAGC------TGA
HM236184.1 O.aries           AACTGTAAAAAGCCATAATT--ATAACAAAAAT--AAATGACGAAAGTAACCCTACAATAGC------TGA
NC 001941.1 O.aries          AACTGTAAAAAGCCATAATT--ATAACAAAAAT--AAATGACGAAAGTAACCCTACAATAGC------TGA
FJ207539.1 R.rupicapra       AACTGTAAAAAGCCATAACT--ATAACAAAAAT--AAATGACGAAAGTAACCCTACAGCAGC------TGA
AY670666.1 R.rupicapra       AACTGTAAAAAGCCATAACT--ATAACAAAAAT--AAATGACGAAAGTAACCCTACAGCAGC------TGA
AM158314.1_R.rupicapra       AACTGTAAAAAGCCATAACT--ATAACAAAAAT--AAATGACGAAAGTAACCCTACAGTAGC------TGA
AJ849535.1_C.hircus          AACTGTAAAAAGCCATAATT--ACAACAAAAAT--AGATGACGAAAGTAACCCTACTGCAGC------TGA
AY184430.1_C.elaphus         AGCTGTAAAAAGCCATAATT--GCAACAAAAAT--AAATAACGAAAGTAACTTTACAGCCGC------TGA
AY184439.1 C.capreolus       AGCTGTAAAAAGCCATAATT--ATAATGAAAAT--AGATAACGAAAGTAACTTTAAAACAGC------TGA
AJ311164.1 A.flavicollis     ATATGTGAAAATTCATTGTTC-GGCCCTAAAAT--CAATAACGAAAGTAGTTCTAATAATTT------TAC
AJ311131.1 A.sylvaticus      ATATGTGAAAATTCATTGTTA-GGACCTAAGCT--CAATAACGAAAGTAGTTCTAATAATTC------TAC
AJ250356.1 C.glareolus       ATATGTGAAAATTCATCGTT--GGACTTAAAAY--CAGTAACGAAAGTAATTCTAATTCACC------TGA
JN711443.1 V.vulpes          AGCCGTAAAAAGCTACAGTT--ACCAT-AAAAT--ATACTACGAAAGTGACTTTAAAATTTT------CTG
EU740412.1 C.l.familiaris    AGCCGTAAAAAGCTACAGTT--ATCAT-AAAAT--AAACCACGAAAGTGACTTTATAATAAT------CTG
AY012102 S.araneus           AGCTGTAGAAAGCAACAGTT--AAAACTAAGAT--ACACTACGAAAGTGACTTTATTACAGC------TGA
AF484935.1 T.philomelos      AGCTGTCATAAGCCCAAGATG-CCCATAAGGCCTCCGTCTTCAAAGAAGATCTTAGAACAACGATTAATTG
JN989561.1_Homo              AGTTGTAAAAAACTCCAGTT--GACACAAAAT---AAACTACGAAAGTGGCTTTAACATATC------TGA
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CERVIDAE_d-loop

                                                       HRM_Ung_DL_F >CGATGGACTAATGACTAATCA

EU436771.1 C.elaphus           TAAAATCGCCCACTCCT----TGTAAT---ATTAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU436777.1 C.elaphus           TAAAATCGCCCACTCCT----TGTAGT---ATTAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU436773.1 C.elaphus           TAAAATCGCCCACTCCT----TGTAGT---ATTAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
AF291887.1 C.elaphus           TAAAATCGCCCACTCCT----TGCAAT---ATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
NC 007704.2 C elaphus          TAAAATCGCCCACTCCT----TGCAAT---ATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
JQ004399.1 C.elaphus           TAAAATCGCCCACCCCT----TGCAAT---ATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU544179.1 C.elaphus           TAAAATCGCCCACCCCT----TGCAAT---ATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
CEU12867 C.elaphus             TAAAATCGCCCACTCCT----TGCAAT---ATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU436781.1 C.elaphus           TAAAATCGCCCACTCCT----TGTAGT---ATTAGACATCTCGATGGACTAATGGCTAATCAGCCCAT-GCTCACACATAACTGT
EU544182.1_C.elaphus           TAAAATCGCCCACTCCT----TGTAGT---ATTAGACATCTCGATGGACTAATGGCTAATCAGCCCAT-GCTCACACATAACTGT
AF291886.1_C_elaphus           TAAAATCGCCCACTTCT----TGTAGT---ATTAGACATCTCGATGGACTAATGGCTAATCAGCCCAT-GCTCACACATAACTGT
EU544183.1_C.elaphus           TAAAATCGCCCACTCCT----TGCAAT---ATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU544185.1 C.elaphus           TAAAATCGCCCACTCCT----TGCAAT---ATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU544184.1 C.elaphus           TAAAATCGCCCACTCCT----TGCAAT---ATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU544180.1 C.elaphus           TAAAATCGCCCACTCCT----TGCAAT---ATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU600308.1 C.capreolus         TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
AY625825.1 C.capreolus         TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU600300.1 C capreolus         TAAAATCGCCCACTCTT----TCCCCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU600300.1 C.capreolus         TAAAATCGCCCACTCTT----TCCCCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU600304.1 C capreolus         TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU600304.1 C.capreolus         TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU600306.1 C.capreolus         TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU600296.1 C.capreolus         TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU600294.1 C.capreolus         TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU600302.1 C capreolus         TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU600310.1 C .capreolus        TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU600312.1 C.capreolus         TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
JN632610.1 C.capreolus         TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU600314.1 C.capreolus         TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
EU600316.1 C capreolus         TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
AY625819.1 C.capreolus         TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
AY625823.1 C.capreolus         TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
X78798.1_S.araneus#            TAAAATCGCCCACTCTT----TCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
AY769263.1_C.russula#          TAAATCCGCCCATTCAT----TCCCCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCCGACACATAACTGT
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AF010406.1 O.aries#            TAAAATCGCCCACTCTT----TCCCCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCCTA-ACATAACTGT
EU887455.1 R rupicapra#        TAGAATCGCCCATTCTT----TCCTCTTAAATAAGACATCTCGATGGACTGGTGACTAATCAGCCCAT-GCTCACACATAACTGT
EU740415.1 C.familiaris#       TATTTACTCCAATCCTACTAATTCTCGCAAATGGGACATCTCGATGGACTAATGACTAATCAGCCCAT-GATCACACATAACTGT
JN711443.1 V.vulpes#           TGTTCACTCCAATCCTACTAATCCTCTCAAATGGGACATCTCGATGGACTAATGACTAATCAGCCCAT-GATCACACATAACTGT
AY588252.1 A.sylvaticus#       CGTTATCGCCCATACGT----TCCCCTTAAATAAGACATCTCGATGGTACGG-GTCTAATCAGCCAAG-ACTTC--CATAACTGT
AY588264.1 A.flavicollis#     CGTTATCGCCCATACGT----TCCCCTTAAATAAGACATCTCGATGGTACGG-GTCTAATCAGCCAAG-ACTTC--CATAACTGT
AF367197.1 C.glareolus#        TTTTATCGTCCATACGT----TCCCCTTAAATAAGACATCTCGATGGTACGG-GTCTAATCAGCCCAT-GCCTA-ACATAACTGT
EU259145.1 C.hircus#           TAAAATCGCCCACTCTT----CCCTCTTAAATAAGACATCTCGATGGACTAATGACTAATCAGCCCAT-GCTCACACATAACTGT
HQ200179.1_H.sapiens           TA-AATAGCCCACACGT----TCCCCTTAAATAAGACATCACGATGGATCACAGGTCTATCACCCTATTAACCACTCACGGGAGC

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EU436777.1 C.elaphus           GGTGTCATACATTTGGTATTTTTAATTTTTGGGGGGA-------TGCTTGGACTCAGCAATGACC---GGCTGGC----GGTCCCG
EU436773.1 C.elaphus           GGTGTCATACATTTGGTATTTTTAATTTTTGGGGGGA-------TGCTTGGACTCAGCAATGACC---GGCTGGC----GGTCCCG
AF291887.1 C.elaphus           GGTGTCATACATTTGGTATTTTTAATTTTTGGGGGGA-------TGCTTGGACTCAGCAATGACC---GTCTGAC----GGTCCTG
NC 007704.2 C elaphus          GGTGTCATACATTTGGTATTTTTAATTTTTGGGGGGA-------TGCTTGGACTCAGCAATGACC---GTCTGGC----GGTCCCG
JQ004399.1 C.elaphus           GGTGTCATACATTTGGTATTTTTAATTTTTGGGGGGA-------TGCTTGGACTCAGCAATGACC---GTCTGGC----GGTCCCG
EU544179.1 C.elaphus           GGTGTCATACATTTGGTATTTTTAATTTTTGGGGGGA-------TGCTTGGACTCAGCAATGACC---GTCTGGC----GGTCCCG
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EU436781.1 C.elaphus           GGTGTCATACATTTGGTATTTTTAATTTTTGGGGGGA-------TGCTTGGACTCAGCAATGACC---GTCTGGC----GGTCCCG
EU544182.1 C.elaphus           GGTGTCATACATTTGGTATTTTTAATTTTTGGGGGGA-------TGCTTGGACTCAGCAATGACC---GTCTGGC----GGTCCCG
AF291886.1_C_elaphus           GGTGTCATACATTTGGTATTTTTAATTTTTGGGGGGA-------TGCTTGGACTCAGCAATGACC---GTCTGGC----GGTCCCG
EU544183.1_C.elaphus           GGTGTCATACATTTGGTATTTTTAATTTTTGGGGGGA-------TGCTTGGACTCAGCAATGACC---GTCTGGC----GGTCCCG
EU544185.1_C.elaphus           GGTGTCATACATTTGGTATTTTTAATTTTTGGGGGGA-------TGCTTGGACTCAGCAATGACC---GTCTGGC----GGTCCCG
EU544184.1 C.elaphus           GGTGTCATACATTTGGTATTTTTAATTTTTGGGGGGA-------TGCTTGGACTCAGCAATGACC---GTCTGGC----GGTCCCG
EU544180.1 C.elaphus           GGTGTCATACATTTGGTATTTTTAATTTTTGGGGGGA-------TGCTTGGACTCAGCAATGACC---GTCTGAC----GGTCCCG
EU600308.1 C.capreolus         GGTGTCATACATTTGGTATTTTTTAATTTTTGGGGGA-------TGCTTGGACTCAGCTATGGCC---GTCAAA-----GGCCCCG
AY625825.1 C.capreolus         GGTGTCATACATTTGGTATTTTTTAATTTTTGGGGGA-------TGCTTGGACTCAGCTATGGCC---GTCAAA-----GGCCCCG
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EU600300.1 C.capreolus         GGTGTCATACATTTGGTATTTTTTAATTTTTGGGGGA-------TGCTTGGACTCAGCTATGGCC---GTCAAA-----GGCCCCG
EU600304.1 C capreolus         GGTGTCATACATTTGGTATTTTTTAATTTTTGGGGGA-------TGCTTGGACTCAGCTATGGCC---GTCAAA-----GGCCCCG
EU600304.1 C.capreolus         GGTGTCATACATTTGGTATTTTTTAATTTTTGGGGGA-------TGCTTGGACTCAGCTATGGCC---GTCAAA-----GGCCCCG
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EU600294.1 C.capreolus         GGTGTCATACATTTGGTATTTTTTAATTTTTGGGGGA-------TGCTTGGACTCAGCTATGGCC---GTCAAA-----GGCCCCG
EU600302.1 C capreolus         GGTGTCATACATTTGGTATTTTTTAATTTTTGGGGGA-------TGCTTGGACTCAGCTATGGCC---GTCAAA-----GGCCCCG
EU600310.1 C .capreolus        GGTGTCATACATTTGGTATTTTTTAATTTTTGGGGGA-------TGCTTGGACTCAGCTATGGCC---GTCAAA-----GGCCCCG
EU600312.1 C.capreolus         GGTGTCATACATTTGGTATTTTTTAATTTTTGGGGGA-------TGCTTGGACTCAGCTATGGCC---GTCAAA-----GGCCCCG
JN632610.1 C.capreolus         GGTGTCATACATTTGGTATTTTTTAATTTTTGGGGGA-------TGCTTGGACTCAGCTATGGCC---GTCAAA-----GGCCCCG
EU600314.1 C.capreolus         GGTGTCATACATTTGGTATTTTTTAATTTTTGGGGGA-------TGCTTGGACTCAGCTATGGCC---GTCAAA-----GGCCCCG
EU600316.1 C capreolus         GGTGTCATACATTTGGTATTTTTTAATTTTTGGGGGA-------TGCTTGGACTCAGCTATGGCC---GTCAAA-----GGCCCCG
AY625819.1 C.capreolus         GGTGTCATACATTTGGTATTTTTTAATTTTTGGGGGA-------TGCTTGGACTCAGCTATGGCC---GTCAAA-----GGCCCCG
AY625823.1 C.capreolus         GGTGTCATACATTTGGTATTTTTTAATTTTTGGGGGA-------TGCTTGGACTCAGCTATGGCC---GTCAAA-----GGCCCCG
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AY769263.1_C.russula#          GGTGTCATACATTTGGTATCTTTAATTTTTTGGGGGGAG-AGCTTGCTATGACTCCGCTAACATTT-AATCTCGCCAATGCAGTTG
AY918370.1_C.leucodon#         GGTGTCATGCGGTTGGTATCTTTAATTTTTAGGGGGGAG-AGCTTGCTATGACTCCACTAACATTT-AATTTCACCAATACAGTTG
AF010406.1_O.aries#            GGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-A-------TGCTTGGACTCAGCTATGGCC---GTCTGA-----GGCCT-G
EU887455.1 R rupicapra#        GCTGTCATACATTTGGTATTTTTTTATTTTGGGGG-A-------TGCTTGGACTCAGCTATGGCC---GTCAAA-----GGCCCCG
EU740415.1 C.familiaris#       GGTGTCATGCATCTGGTATCTTTTAATTTTTAGGGGGGG-AATCTGCTATCACTCACCTACGACC---GCAACGGCACTAACTCTA
JN711443.1 V.vulpes#           GGTGTCATGCATTTGGTATCTTTTTTTTTTGGGGGGGGGGAACTTGCTATCACTCAGCTATGACC---GCAACGGCACTAACTCTA
AY588252.1 A.sylvaticus#       GGTCTCGAGCAGTTGGTATTTTTTTATTTTTAGG--A-------TGCTGTGACTCAGC-ATAGCC---GTCAAG-------GCATG
AY588264.1 A.flavicollis#     GGTCTCGGGCAGTTGGTATTTTTTTATTTTTAGG--A-------TGCTGTGACTCAGC-ATAGCC---GTCAAG-------GCATG
AF367197.1 C.glareolus#        GGTCTCGGGCAGTTGGTATTTTTTAATTTTCGGG--A-------TGGTATCACTCAGC-ATAGCC---GTCAAG-------GCATG
EU259145.1 C.hircus#           GCTGTCATACATTTGGTATTTTTTAATTTTCGGGG-A-------TGCTTGGACTCAGCTATGGCC---GTCTGA-----GGCCCCG
HQ200179.1_H.sapiens           TCT-CCATGCATTTGGTATTTTCG---TCTGGGGGGTATGCACGCGATAGCATTGCGGGACGCTG-GAGCCGGAGCACC--CTATG
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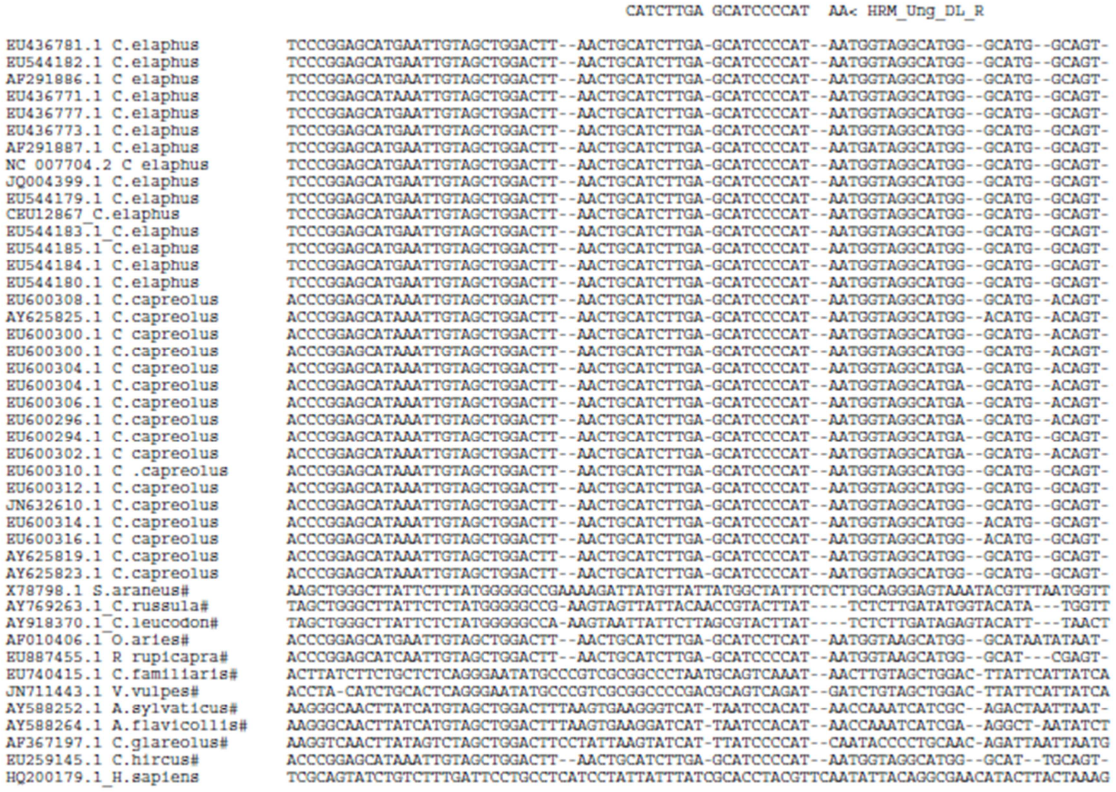

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EU544182.1 C.elaphus          TCCCGGAGCATGAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
AF291886.1 C elaphus          TCCCGGAGCATGAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
EU436771.1 C.elaphus          TCCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
EU436777.1 C.elaphus          TCCCGGAGCATGAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
EU436773.1 C.elaphus          TCCCGGAGCATGAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
AF291887.1 C.elaphus          TCCCGGAGCATGAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGATAGGCATGG--GCATG--GCAGT-
NC 007704.2 C elaphus         TCCCGGAGCATGAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
JQ004399.1 C.elaphus          TCCCGGAGCATGAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
EU544179.1 C.elaphus          TCCCGGAGCATGAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
CEU12867_C.elaphus            TCCCGGAGCATGAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
EU544183.1_C.elaphus          TCCCGGAGCATGAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
EU544185.1 C.elaphus          TCCCGGAGCATGAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
EU544184.1 C.elaphus          TCCCGGAGCATGAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
EU544180.1 C.elaphus          TCCCGGAGCATGAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
EU600308.1 C.capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--ACAGT-
AY625825.1 C.capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--ACATG--ACAGT-
EU600300.1 C capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--ACAGT-
EU600300.1 C.capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--ACAGT-
EU600304.1 C capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGA--GCATG--ACAGT-
EU600304.1 C.capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGA--GCATG--ACAGT-
EU600306.1 C.capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGA--GCATG--ACAGT-
EU600296.1 C.capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGA--GCATG--ACAGT-
EU600294.1 C.capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGA--GCATG--GCAGT-
EU600302.1 C capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGA--GCATG--ACAGT-
EU600310.1 C .capreolus       ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
EU600312.1 C.capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
JN632610.1 C.capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
EU600314.1 C.capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--ACATG--GCAGT-
EU600316.1 C capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--ACATG--GCAGT-
AY625819.1 C.capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
AY625823.1 C.capreolus        ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCATG--GCAGT-
X78798.1 S.araneus#           AAGCTGGGCTTATTCTTTATGGGGGCCGAAAAGATTATGTTATTATGGCTATTTCTCTTGCAGGGAGTAAATACGTTTAATGGTT
AY769263.1_C.russula#         TAGCTGGGCTTATTCTCTATGGGGGCCG-AAGTAGTTATTACAACCGTACTTAT----TCTCTTGATATGGTACATA---TGGTT
AY918370.1_C.leucodon#        TAGCTGGGCTTATTCTCTATGGGGGCCA-AAGTAATTATTCTTAGCGTACTTAT----TCTCTTGATAGAGTACATT---TAACT
AF010406.1 O.aries#           ACCCGGAGCATGAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCTCAT--AATGGTAAGCATGG--GCATAATATAAT-
EU887455.1 R rupicapra#       ACCCGGAGCATCAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAAGCATGG--GCAT---CGAGT-
EU740415.1 C.familiaris#      ACTTATCTTCTGCTCTCAGGGAATATGCCCGTCGCGGCCCTAATGCAGTCAAAT--AACTTGTAGCTGGAC-TTATTCATTATCA
JN711443.1 V.vulpes#          ACCTA-CATCTGCACTCAGGGAATATGCCCGTCGCGGCCCCGACGCAGTCAGAT--GATCTGTAGCTGGAC-TTATTCATTATCA
AY588252.1 A.sylvaticus#      AAGGGCAACTTATCATGTAGCTGGACTTTAAGTGAAGGGTCAT-TAATCCACAT--AACCAAATCATCGC--AGACTAATTAAT-
AY588264.1 A.flavicollis#     AAGGGCAACTTATCATGTAGCTGGACTTTAAGTGAAGGATCAT-TAATCCACAT--AACCAAATCATCGA--AGGCT-AATATCT
AF367197.1 C.glareolus#       AAGGTCAACTTATAGTCTAGCTGGACTTCCTATTAAGTATCAT-TTATCCCCAT--CAATACCCCTGCAAC-AGATTAATTAATG
EU259145.1 C.hircus#          ACCCGGAGCATAAATTGTAGCTGGACTT--AACTGCATCTTGA-GCATCCCCAT--AATGGTAGGCATGG--GCAT--TGCAGT-
HQ200179.1_H.sapiens          TCGCAGTATCTGTCTTTGATTCCTGCCTCATCCTATTATTTATCGCACCTACGTTCAATATTACAGGCGAACATACTTACTAAAG
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Table A1 Identification results by HRMA and sequence BLASTn for questing ticks bloodmeal analysis during Real-time HRMA optimization; Tm, melting temperature; Tm1, melting temperature peak 1; Tm2, melting temperature peak 2.continued in the next page.

		Muroidea			**Soricidae**				**Passeriformes**			
	sample	Tm °C			Tm °C (n°rep)				Tm °C (n°rep)			
SITE	**name**	(n°rep)	HRMA genotype	BLAST genotype	Tm1	Tm2	HRMA genotype	BLAST genotype	Tm1	Tm2	HRMA genotype	BLAST genotype
CON	1_CO	74.2 (1)	out of range	no sequences obtained-too short					79.6		no match-out of range	148 bp-no similarity found
	2_CO								81.5		no match-out of range	103 bp-Ixodes sp
	3_CO								82.8	86.6	no match-out of range	575 bp-Ixodes sp
	4_CO											
	5_CO								82.1		no match-out of range	346 bp- no similarity found
	6_CO											
	7_CO											
	8_CO											
	9_CO											
	10_CO											
	11_CO				77.0	70.4 (1)	out of range					
	12_CO	81.9 (2)	*Apodemus sp.*	*A.flavicollis* 100%	81.8	74.4 (1)	*C. suaveolens*	*C. russula 99%*[a]				
	13_CO				83.1 (1)		out of range					
TRA	1_TR								83.6		strange profile	123 bp-Ixodes sp
	2_TR											
	3_TR								78.6	84.4	strange profile	154 bp double peaks-T.merula 99% NO POSITIVE
	4_TR								79.5	84.9	strange profile	double peak-not readable sequences
	5_TR											
	6_TR											
	7_TR											
	8_TR				81.2	83.9 (1)	late amplification-strange profile	no sequence obtained				
	9_TR											
	10_TR				81.6 (1)		*C. suaveolens*	*C. russula 97%*[a]				
	11_TR				82.4 (1)		*C. leucodon*	*C. leucodon* 99%				
	12_TR				81.9 (1)		*C. leucodon ??*	*C. leucodon* 99%				
CAD	1_CA								79.8	82.0	out of range	219 bp-Ixodes sp
	2_CA											
	3_CA											
	4_CA											
	5_CA											
	6_CA											
	7_CA											
	8_CA				82.3	75.3 (1)	*C. leucodon?*-strange profile	*C. leucodon* 99%, only F primer				
	9_CA	78.7	out of range	no sequences obtained-too short								
	10_CA				81.6	74.4 (1)	*C. suaveolens*	*C. russula 98%*[a]	58.1	84.3	late amplification-strange profile	double peak in the sequence-no readable
	11_CA											
	12_CA											
	13_CA											
	14_CA				80.5 (1)		*Sorex sp*	*S. antinorii* 100%				
PIE	1_PI	81.5 (1)	*Apodemus sp.*	*A. flavicollis* 100%								
	2_PI											
	3_PI				86.2 (1)		out of range	261 bp-Ixodes spp				
	4_PI											
	5_PI	81.3 (1)	*M. glareolus*	*A. flavicollis* 98% (2 del)	83.7 (1)		out of range	no sequences assembly				
	6_PI											
	7_PI				82.3 - 74.4 (1)		*C. leucodon*	*C . leucodon* 100%				
	8_PI				82.3 - 74.0 (1)		*C. leucodon*	*C . leucodon* 100%				
	9_PI				81.7 - 73.7 (1)		*C. suaveolens*	*C. russula 98%*[a]				
	10_PI				82.4 (1)		*C. leucodon*	*C . leucodon* 100%	84.1		late amplification-strange profile	
	11_PI				81.4 (1)		*C. suaveolens*	*C. russula 99%*[a]				
	12_PI				82.3 - 73.6 (1)		*C. leucodon*	*C . leucodon* 100%				
	13_PI				80.5 (1)		*Sorex sp*	*S. antinorii* 99%				

Table A1 continued

SITE	sample name	Canidae				Caprinae					Cervidae				Mixed bloodmeal
		Tm °C (replicate		HRMA genotype	BLAST genotype	Tm °C (n°rep)			HRMA genotype	BLAST genotype	Tm °C (n°rep)		HRMA genotype	BLAST genotype	
		Tm1	Tm2			Tm1	Tm2	Tm3			Tm1	Tm2			
CON	1_CO	79.9 (1)		*C.l. familiaris*	*C.l. familiaris* 100%	80.7	83.9	88.2 (1)	no match-strange profile	double sequences not readable			-		
	2_CO	80.4 (2)		*C.l. familiaris*	*C.l. familiaris* 100%	80.8	83.9 (2)		no match-out of range	*Bos taurus* 99%			-		yes
	3_CO	80.8 (1)		*C.l. familiaris*	*C.l. familiaris* 100%	81.0	84.1 (2)		no match-out of range	*Bos taurus* 99%			-		yes
	4_CO												-		
	5_CO	80.4 (2)		*C.l. familiaris*	*C.l. familiaris* 100%	80.8	83.7 (2)		out of range	*Bos taurus* 99%			-		yes
	6_CO	80.5	82.7	no match-strange profile	no similarity found						83.5	80.7 (1)	*C. elaphus*	*C. elaphus* 100%	
	7_CO	80.2 (2)		*C.l. familiaris*	*C.l. familiaris* 100%								-		
	8_CO												-		
	9_CO	80.6 (1)		*C.l. familiaris*	*C.l. familiaris* 100%								-		
	10_CO										82.7	80.6 (2)	*C. capreolus*	*C. capreolus* 100%	
	11_CO												-		
	12_CO												-		yes
	13_CO												-		
TRA	1_TR	80.7 (1)		*C.l. familiaris*	*C.l. familiaris* 100%	74.3	83.7	87.9	no match-out of range	double sequences not readable	83.5	80.7 (1)	*C. elaphus*	*C. elaphus* 100%	
	2_TR	80.9 (2)		*C.l. familiaris*	*C.l. familiaris* 100%								-		
	3_TR	85.1		no match-out of range	no similarity found - Ixodes sp						83.6	80.7 (1)	*C. elaphus*	*C. elaphus* 100%	
	4_TR	80.3 (1)		*C.l. familiaris*	*C.l. familiaris* 100%										
	5_TR	83.4	80.7 (1)	*V. vulpes*	*V. vulpes* 100%										
	6_TR														
	7_TR														
	8_TR														
	9_TR														
	10_TR														
	11_TR	83.7	87.3 (1)	*V. vulpes*	*V. vulpes* 100%										yes
	12_TR														
CAD	1_CA	80.7		strange derivative profile	double sequences not readable	78.3	80.8	84.0	no match-out of range	double sequences not readable					
	2_CA					73.5	83.8	88.2	no match-out of range	double sequences not readable					
	3_CA	80.6		strange derivative profile	no similarity found						83.2		no match-strang	Ixodes sp.	
	4_CA	78.5	80.2	no match-strange profile	no similarity found						83.6	80.8 (1)	*C. elaphus*	*C. elaphus* 100%	
	5_CA														
	6_CA														
	7_CA										82.5	80.5 (2)	*C. capreolus*	*C. capreolus* 100%	
	8_CA														
	9_CA														
	10_CA														
	11_CA														
	12_CA	82.8		no match-strange profile	no similarity found										
	13_CA														
	14_CA														
PIE	1_PI			-							83.4	80.5 (2)	*C. elaphus*	*C. elaphus* 100%	yes
	2_PI	80.7	82.7	no match- strange profile	248 bp - no similarity found										
	3_PI	80.4 (2)		*C.l. familiaris*	*C.l. familiaris* 100%										
	4_PI			-							83.6	80.7 (2)	*C. elaphus*	*C. elaphus* 100%	
	5_PI			-											
	6_PI			-							83.5	80.6 (1)	*C. elaphus*	*C. elaphus* 100%	
	7_PI														
	8_PI										83.3	80.5 (1)	*C. elaphus*	*C. elaphus* 99%	yes
	9_PI														
	10_PI														
	11_PI														
	12_PI														
	13_PI														

FOOTNOTES Table A1:

[a] for C. suaveolens sequences BLAST give as maximum identity *C. russula*, because no sequence of *C. suaveolens d-loop* is available in the GenBank database; 100% C. suaveolens, after alignment with sequence obtained from DNA tissue samples of *C. suaveolens* by authors.

Appendix 2 *Ovis* spp. alignment and BLASTn results

ClustalX 2.0.12 alignment of *Ovis* spp sequences retrieved from GenBank and sequences otained with HRM_Cer primers from questing nymphs (IRQxxxxx) and from the engorged ticks collected while feeding on a mouflon (IRH000213). A *C. capreolus* and a *C. elaphus* sequence from questing nymphs were also included.

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                                     ************* ******* *******  * ******* *** ****** ****** ******* * * *  **** *************** *** ** **     * ** * **********  *********** ********* ***********  ***
            Z35264.1_O.aries AGACATCTCGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACAGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCCGACCCGGAGCATGAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCTCATAATGGTAA
            Z35258.1_O.aries AGACATCTCGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACAGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCCGACCCGGAGCATGAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCTCATAATGGTAA
  GU350335.1_O.aries_musimon AGACATCTCGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACTGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCTGACCCGGAGCATGAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCTCATAATGGTAA
            Z35240.1_O.aries AGACATCTCGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACAGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCCGACCCGGAGCATGAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCTCATAATGGTAA
            Z35233.1_O.aries AGACATCTCGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACAGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCCGACCCGGAGCATAGATTGTAGCTGGACTTAACTGCATCTTGAGCATCCTCATAATGGTAA
            Z35267.1_O.aries AGACATCTCGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACAGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCCGACCCGGAGCATGAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCTCATAATGGTAA
  GU350328.1_O.aries_musimon AGACATCTCGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACTGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCCGACCCGGAGCATGAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCCCATAATGGTAA
            IRQ52513_HRM_Cer --------CGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACTGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCCGACCCGGAGCATGAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCCCATA-------
            IRQ52013_HRM_Cer --------CGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACTGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG--AGGCCCGACCCGGAGCATGAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCCCAT--------
  AF039579.1_O.aries_musimon AGACATCTCGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACTGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG--AGGCCCGACCCGGAGCATGAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCCCATAATGGTAA
  AY091487.1_O.aries_musimon AGACATCTCGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACTGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCCGACCCGGAGCATGAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCCCATAATGGTAA
  HM236184.1_O.aries_musimon AGACATCTCGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACTGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCCGACCCGGAGCATGAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCCCATAATGGTAA
            IRQ15013_HRM_Cer --------CGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACTGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCCGACCCGGAGCATGAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCCCAT--------
           IRH000213_mouflon --------CGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACTGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCCGACCCGGAGCATGAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCCCAT--------
         IRQ071212_A_HRM_Cer --------CGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACTGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCCGACCCGGAGCATGAATTGTAGCTGGACTTAACTGCCTCTTGAGCATCCCCAT--------
           IRQ059412_HRM_Cer --------CGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACTGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCCGACCCGGAGCATAAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCCCATA-------
   KF312238.1_O.aries_ophion AGACATCTCGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACTGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCCGACCCGGAGCATAAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCTCATAATGGTAA
            Z35249.1_O.aries AGACATCTCGATGGACTAATGACTAATCAGCCCATGCCTA-ACATAACAGTGGTGTCATGCATTTGGTATTTTTTAATTTTTGGGG-ATGCTTGGACTCAGCTATGGCCGTCTG-AGGCCCCGACCCGGAGCATGAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCTCATAATGGTAA
          AB006801.1_O.aries AGCCATCTCGATGGACTAATGGCTAATCAACCCATGCTCACACATAACTGTGCTGTCATACATTTGGTATTTTTTAATGTCTGGGG-ATGCTTGGACTCAGCTATGACCATCTG-AGGCCCTGACCCGGAGCATGTATTGTAGCTGGGCTTAACTGCATCTTGAGCATCACCATAATGGTAG
IRQ02713 HRM Cer C.capreolus --------CGATGGACTAATGACTAATCAGCCCATGCTCACACATAACTGTGGTGTCATACATTTGGTATTTTTTAATTTTTGGGGGATGCTTGGACTCAGCTATGGCCGTCAA-AGGCCCCGACCCGGAGCATAAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCCCAT--------
 IRQ039412_HRM_Cer_C.elaphus --------CGATGGACTAATGACTAATCAGCCCATGCTCACACATAACTGTGGTGTCATACATTTGATATTTTTAATTTTTGGGGGGATGCTTGGACTCAGCAATGACCGTCTGGAGGTCCCGTCCCGGAGCATGAATTGTAGCTGGACTTAACTGCATCTTGAGCATCCCCATAA------
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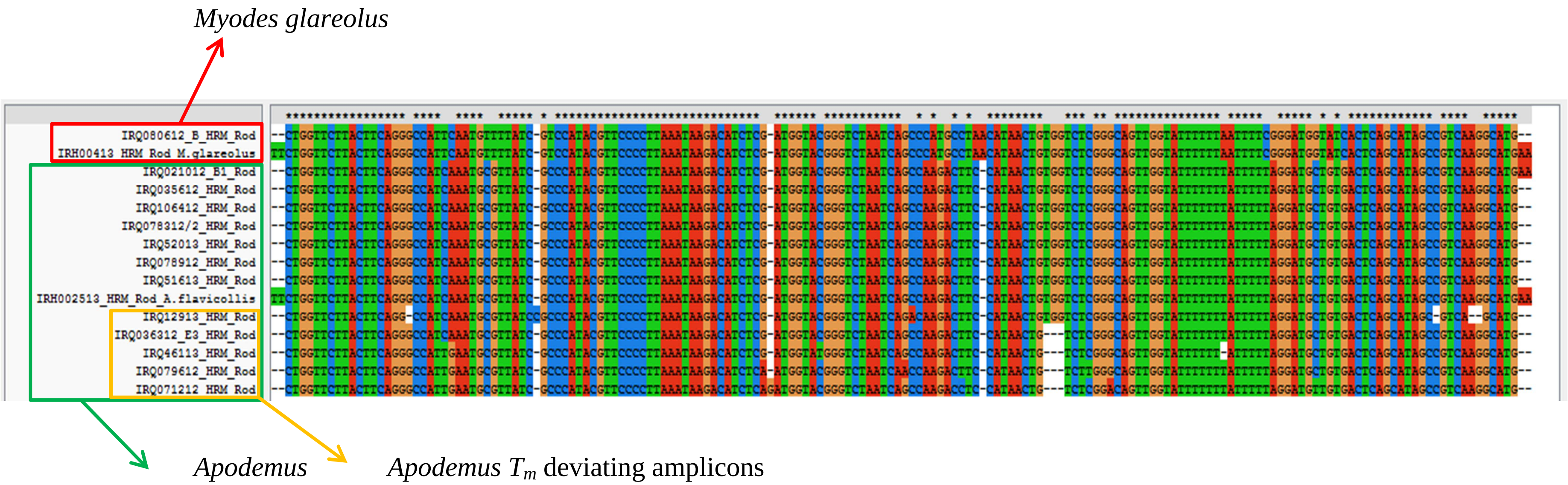
BLASTn results of HRM_Cer *Ovis* spp amplicons

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

For 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*.

Appendix 3 Sequences alignment of HRM_Rod amplicons

Sequences alignment of HRM_Rod amplicons with special reference to deviating HRMA *Apodemus* spp. amplicons. IRHxxxx control samples sequences; IRQxxxx questing nymphs sequences.



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                                  ***************** ****  ****  ***** * *****************************  ****** ***********  * *  * *  ********   *** ** *************** *****  ***** * * ************ ****  *****
            IRQ080612_B_HRM_Rod --CTGGTTCTTACTTCAGGGCCATTCAATGTTTTATC-GTCCATACGTTCCCCTTAAATAAGACATCTCG-ATGGTACGGGTCTAATCAGCCCATGCCTAACATAACTGTGGTCTCGGGCAGTTGGTATTTTTTAATTTTCGGGATGGTATCACTCAGCATAGCCGTCAAGGCATG--
   IRH00413 HRM Rod M.glareolus TTCTGGTTCTTACTTCAGGGCCATTCAATGTTTTATC-GTCCATACGTTCCCCTTAAATAAGACATCTCG-ATGGTACGGGTCTAATCAGCCCATGCCTAACATAACTGTGGTCTCGGGCAGTTGGTATTTTTTAATTTTCGGGATGGTATCACTCAGCATAGCCGTCAAGGCATGAA
               IRQ021012_B1_Rod --CTGGTTCTTACTTCAGGGCCATCAAATGCGTTATC-GCCCATACGTTCCCCTTAAATAAGACATCTCG-ATGGTACGGGTCTAATCAGCCAAGACTTC-CATAACTGTGGTCTCGGGCAGTTGGTATTTTTTTATTTTTAGGATGCTGTGACTCAGCATAGCCGTCAAGGCATGAA
              IRQ035612_HRM_Rod --CTGGTTCTTACTTCAGGGCCATCAAATGCGTTATC-GCCCATACGTTCCCCTTAAATAAGACATCTCG-ATGGTACGGGTCTAATCAGCCAAGACTTC-CATAACTGTGGTCTCGGGCAGTTGGTATTTTTTTATTTTTAGGATGCTGTGACTCAGCATAGCCGTCAAGGCATG--
              IRQ106412_HRM_Rod --CTGGTTCTTACTTCAGGGCCATCAAATGCGTTATC-GCCCATACGTTCCCCTTAAATAAGACATCTCG-ATGGTACGGGTCTAATCAGCCAAGACTTC-CATAACTGTGGTCTCGGGCAGTTGGTATTTTTTTATTTTTAGGATGCTGTGACTCAGCATAGCCGTCAAGGCATG--
            IRQ078312/2_HRM_Rod --CTGGTTCTTACTTCAGGGCCATCAAATGCGTTATC-GCCCATACGTTCCCCTTAAATAAGACATCTCG-ATGGTACGGGTCTAATCAGCCAAGACTTC-CATAACTGTGGTCTCGGGCAGTTGGTATTTTTTTATTTTTAGGATGCTGTGACTCAGCATAGCCGTCAAGGCATG--
               IRQ52013_HRM_Rod --CTGGTTCTTACTTCAGGGCCATCAAATGCGTTATC-GCCCATACGTTCCCCTTAAATAAGACATCTCG-ATGGTACGGGTCTAATCAGCCAAGACTTC-CATAACTGTGGTCTCGGGCAGTTGGTATTTTTTTATTTTTAGGATGCTGTGACTCAGCATAGCCGTCAAGGCATG--
              IRQ078912_HRM_Rod --CTGGTTCTTACTTCAGGGCCATCAAATGCGTTATC-GCCCATACGTTCCCCTTAAATAAGACATCTCG-ATGGTACGGGTCTAATCAGCCAAGACTTC-CATAACTGTGGTCTCGGGCAGTTGGTATTTTTTTATTTTTAGGATGCTGTGACTCAGCATAGCCGTCAAGGCATG--
               IRQ51613_HRM_Rod --CTGGTTCTTACTTCAGGGCCATCAAATGCGTTATC-GCCCATACGTTCCCCTTAAATAAGACATCTCG-ATGGTACGGGTCTAATCAGCCAAGACTTC-CATAACTGTGGTCTCGGGCAGTTGGTATTTTTTTATTTTTAGGATGCTGTGACTCAGCATAGCCGTCAAGGCATG--
IRH002513_HRM_Rod_A.flavicollis TTCTGGTTCTTACTTCAGGGCCATCAAATGCGTTATC-GCCCATACGTTCCCCTTAAATAAGACATCTCG-ATGGTACGGGTCTAATCAGCCAAGACTTC-CATAACTGTGGTCTCGGGCAGTTGGTATTTTTTTATTTTTAGGATGCTGTGACTCAGCATAGCCGTCAAGGCATGAA
               IRQ12913_HRM_Rod --CTGGTTCTTACTTCAGG-CCATCAAATGCGTTATCCGCCCATACGTTCCCCTTAAATAAGACATCTCG-ATGGTACGGGTCTAATCAGACAAGACTTC-CATAACTGTGGTCTCGGGCAGTTGGTATTTTTTTATTTTTAGGATGCTGTGACTCAGCATAGC-GTCA--GCATG--
           IRQ036312_E3_HRM_Rod --CTGGTTCTTACTTCAGGGCCATCAAATGCGTTATC-GCCCATACGTTCCCCTTAAATAAGACATCTCG-ATGGTACGGGTCTAATCAGCCAAGACTTC-CATAACTG---TCTCGGGCAGTTGGTATTTTTTTATTTTTAGGATGCTGTGACTCAGCATAGCCGTCAAGGCATG--
               IRQ46113_HRM_Rod --CTGGTTCTTACTTCAGGGCCATTGAATGCGTTATC-GCCCATACGTTCCCCTTAAATAAGACATCTCG-ATGGTATGGGTCTAATCAGCCAAGACTTC-CATAACTG---TCTCGGGCAGTTGGTATTTTTT-ATTTTTAGGATGCTGTGACTCAGCATAGCCGTCAAGGCATG--
              IRQ079612_HRM_Rod --CTGGTTCTTACTTCAGGGCCATTGAATGCGTTATC-GCCCATACGTTCCCCTTAAATAAGACATCTCA-ATGGTACGGGTCTAATCAACCAAGACTTC-CATAACTG---TCTTGGGCAGTTGGTATTTTTTTATTTTTAGGATGCTGTGACTCAGCATAGCCGTCAAGGCATG--
              IRQ071212 HRM Rod --CTGGTTCTTACTTCAGGGCCATTGAATGCGTTATC-GCCCATACGTTCCCCTTAAATAAGACATCTCAGATGGTACGGGTCTAATCAGCCAAGACCTC-CATAACTG---TCTCGGACAGTTGGTATTTTTTTATTTTTAGGATGTTGTGACTCAGCATAGCCGTCAAGGCATG--
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Myodes glareolus

Apodemus

Apodemus T_m deviating amplicons