UNIVERSITY OF VETERINARY MEDICINE

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Molecular analyses of ticks and tick-borne pathogens from Switzerland

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CONTENTS

1. Introduction and aims

In Europe, hard ticks (Acari: Ixodidae) are among the most important transmitters (vectors) of pathogens causing diseases in domestic or wild animals and humans (Jongejan and Uilenberg, 2004). While in the era of climate change the increasing significance of ticks and tick-borne diseases has long been recognized on this continent (Parola and Raoult, 2001), not all regions are equally affected. The most dramatic emergence of new tick species and/or tick-borne pathogens has been witnessed in northern Europe (Mysterud et al., 2017), but relevant changes are also evident in the Mediterranean region (Efstratiou and Karanis, 2019), highlighting the importance of studies monitoring this situation.

Switzerland occupies an intermediate position between western, central and southern Europe. Although it has a temperate climate, this shows considerable variation within the country, ranging from cold weather in high altitudes of the Alps to warmer seasons in its southernmost canton (Ticino) which reaches into the Mediterranean region. Accordingly, the tick fauna of Switzerland reflects the simultaneous presence of species with diverse ecological needs, including cold tolerant tick species (such as *Ixodes ricinus*: Dautel and Knülle, 1997) as well as thermophilic ones in the south (i.e. *Rhipicephalus sanguineus* sensu lato and *Rh. turanicus*: *Aeschlimann et al., 1965; Bernasconi et al., 2002*). In addition, migratory birds may import exotic tick species (Papadopoulos et al., 2002), and may also be responsible for genetic exchange of indigenous tick species between their populations within and outside Switzerland (Lommano et al., 2014).

While the occurrence of tick-borne bacteria and protozoan parasites are well-documented and have recently been updated several times in Switzerland (Casati et al., 2006; Lommano et al., 2012; Hofmann-Lehmann et al., 2016; Oechslin et al., 2017; Pilloux et al., 2018), this epidemiological situation might change rapidly due to emergence of new species. On the other hand, investigations of the genetic diversity and geographical relatedness of tick species in this country appear to be outdated, because Switzerland was not included in the pan-European survey of *I. ricinus* populations (Noureddine et al., 2011), and regional studies date back to around two decades ago (Delaye et al., 1997; de Meeûs et al. 2002).

During the present study, ixodid ticks were collected from various host species in southern Switzerland and consequently analyzed taxonomically, according to mitochondrial markers. With this, the primary aim was to compensate for the above scarcity of the haplotype-related data on tick species occurring in Switzerland. Special emphasis was laid on investigating the possible presence of *I. inopinatus* which can be best identified molecularly (Hauck et al., 2019). It was also within the scope of this study to attempt the detection of rare tick-borne pathogens which have not hitherto been reported in the country or in a particular sample type. These included bacteria (*Borrelia miyamotoi*, *Occidentia massiliensis*) and protozoan parasites (trypanosomes). In the latter analyses, rodent tissues (collected in 2006) were also screened retrospectively.

2. Literature Review

2.1 Ticks species present in Switzerland:

Based on literature data there are three tick species with high veterinary-medical significance in Switzerland. The most important is *Ixodes ricinus*, which is the most widespread hard tick species in Europe. It has a significant role in the transmission of several diseases like Lyme borreliosis, babesiosis, and tick-borne encephalitis. The morphology of *Ixodes* species is characteristic: *Ixodes* have no eyes, the anal groove is anterior to the anus and it has long mouthparts. It can increase in size to 11mm long when it is engorged of blood. *Ixodes ricinus* has a 3-host life cycle meaning that all stages of development feed on different hosts. This hard tick species is widespread in all parts of Europe though less present in the Mediterranean area because the temperature is too high. The high density of this tick species has also increased the risk of pathogen transmission (Beugnet & Marie, 2009). The spreading of the *Ixodes* species is due to several factors including the traveling of its hosts and the climate changes (Beugnet & Marie, 2009). *Ixodes ricinus* has had a role as a vector for a very long time in Switzerland (Rosenberg et al., 1979).

The second most important hard tick species in Switzerland is *Rhipicephalus sanguineus*. It is also called the brown dog tick. *Rhipicephalus sanguineus* is adapted to warmer and tropical environment. That is why it is mostly present in the southern part of Switzerland, Ticino. It is mainly responsible for transmitting the causative agent of Mediterranean spotted fever (Bernasconi et al., 1997, 2002). It has a special basis capituli form that is hexagonal and has eyes. It also has a 3-host life cycle. Although it usually feeds on the same species, it has a different host individual during each stage (the preferred host is the dog). *Rhipicephalus sanguineus* has been seen transported with dogs around Europe and spreading more North than where it was previously found (Beugnet & Marie, 2009).

The third tick with importance is *Dermacentor reticulatus*, the ornate cow tick or meadow tick. It is also present in Switzerland but to a smaller scale. Fewer reports mention this species (Eichenberger et al., 2015), for instance in the western part of Switzerland, around the lake of Geneva. This tick is mostly thermophilic. It is most often observed in continental climates. *Dermacentor reticulatus* nowadays is found all over Europe, although previously it wasn't present in the colder, northern parts. The spreading of the tick species led to an increase of the babesiosis in Europe including Switzerland (Beugnet & Marie, 2009). Some of its specimens were collected in eastern Switzerland (Eichenberger et al., 2015). Like for the other species, the spreading of *Dermacentor* is linked to hosts (dogs) transported around Europe and possibly also climate changes. Like *Rh. sanguineus* it has a 3-host life cycle. *Dermacentor reticulatus* is a vector of several pathogens: *Babesia canis*, *Coxiella burnetii*, several *Rickettsia* species, and others.

A somewhat less important tick species is *Ixodes hexagonus* which is mentioned in few reports only (Eichenberger et al., 2015). This species of tick is also known as the hedgehog tick because its

main host is the European hedgehog. *Ixodes hexagonus* is also an important vector of Lyme disease. Only 2 nymphs of *I. hexagonus* were recorded on a *Pica* (magpie) in the canton Vaud (Papadopoulos et al., 2001). The second less important tick species is *Ixodes trianguliceps*. According to some data (Eichenberger et al., 2015) the second most common tick species found on cats in Switzerland was *I. trianguliceps* but it wasn't mentioned in any other research. It is also very common on rodents. Some other species are mentioned in studies more rarely and more specifically on birds: *Ixodes festai:* found of birds in the Italian part of Switzerland (Ticino). *Ixodes arboricola*: found on different animals in different parts of Switzerland. The scientists also collected immature ticks from nests of *Parus major* (Papadopoulos et al., 2001). *Ixodes lividus* was mostly collected on Sand martins and in their nests. It is the only host of this tick species (Papadopoulos et al., 2001). *Ixodes caledonicus* is a rare tick species that was found only two times in Switzerland, in 1974 and in 1990 (Papadopoulos et al., 2001). *Ixodes frontalis*: a small number of ticks were collected in Ticino in the year 1990. *Haemaphysalis punctata*: only two nymphs were collected during the year 1982 and the year 1990. *Hyaloma marginatum*: this tick species was found on several migrating birds. *Rhipicephalus simus* is rare and only one single tick was collected in the year 1969 on a House martin (Papadopoulos et al., 2001). *Argas reflexus*, a soft tick species has been collected mostly from *Columba livia domestica* (domestic pigeons) in different parts of Switzerland.

2.2 Tick-borne pathogens in Switzerland

Ticks can transmit bacteria, viruses and protozoa like babesia. According to the literature data the most common tick-borne bacteria in Switzerland belong to the genus *Borrelia*. Lyme borreliosis is the most widespread disease transmitted by ticks in Europe. Lyme borreliosis is caused by *Borrelia burgdoferi sensu lato*. It is present in Switzerland (Bernasconi et al., 1997). There are 10 species within *Borrelia burgdoferi sensu lato*; *B. afzelii, B. burgdoferi sensu stricto, B. garinii, B. valaisiana, B. spielmanni, B. bavariensis, B. bissettii, B. finlandensis* (Lommano et al., 2012). Most of these species are present in Switzerland and are detected in the hard tick species *I. ricinus*. Almost all species of the *Borrelia* genus are pathogens that cause diseases in humans. Three of them are among the most frequently detected pathogens in humans; *B. burgdoferi ss, B. garnii,* and *B. afzelli* (Hügli et al., 2009). These bacteria (*Borrelia burgdoferi sensu lato*) can cause different symptoms varying from a rash to severe arthritis, neurological and cardiac problems. *Borrelia* species that are pathogenic for humans are divided into three major phylogenetic groups: Lyme borreliosis, the relapsing fever (in which *B. miyamotoi* belongs), and the reptile associated borreliae. (Kiewra et al., 2014).

Regarding the first two groups of spirochetes: the first group causes Lyme disease and is transmitted by hard ticks (this disease is prevalent all around Europe, Asia, and North America). The second group is the one that causes severe relapsing fever and is transmitted by both soft and hard ticks. *Borrelia miyamotoi* belongs to the relapsing fever group. *Borrelia miyamotoi* is part of the family that is called the relapsing fever group that is usually transmitted by soft-bodied ticks or lice, but *B. miyamotoi* is transmitted by hard ticks. Altogether 6 *Ixodes* tick species (Krause et al., 2015) in North America and Eurasia transmit *B. miyamotoi*. This relapsing fever spirochete has only been identified as a human pathogen recently (Wagemakers et al., 2015). The first time they discovered the pathogen was in *I. persulcatus* ticks in Japan. The pathogen was named after Kenji Miyamoto the entomologist who discovered the pathogen in the ticks. The first time the pathogen *Borrelia miyamotoi* was isolated in Japan was in the year 1995 (Cosson et al., 2014) from the tick species *I. persulcatus* and isolated from the blood of *Apodemus argenteus mice*. From the year 1995 when it was found it has been discovered in various *Ixodes species* in the Northern Hemisphere (USA, Europe), mainly *Ixodes ricinus, I. scapularis, I. pacificus,* and *I. persulcatus* (Cosson et al., 2014). It has also been discovered in the tick species *I. persulcatus* and *I.ricinus* in Russia. (Platonov et al., 2011). But direct proof that the larvae of these tick species can transmit *Borrelia miyamotoi* is not yet established (Wagemakers et al., 2015). The pathogen has been found in the blood of different mammalian reservoir hosts: mice (*Peromyscus leukopus, Apodemus argenteus,* and *A. speciosus*) and voles (*Myodes rutilus, Myodes rufocanus,* and *M. glareolus*). *Peromyscus leucopus* is present in the United States and *Apodemus argentus* in Japan. Both of these species are potential reservoir hosts. In Europe, *Myodes glareolus* appears to be the main reservoir (Cosson et al., 2014). In the more recent years, the pathogen has been found in Europe, Russia, and also in the USA in *Ixodes* ticks.

In 2011 the first human case of *B. miyamotoi* infection was diagnosed in Russia and recently more human cases have been reported in the USA and Netherlands. (Cosson et al., 2014). *Borrelia miyamotoi* and *B. burgdorferi* (and other LB tick species) have the same tick vectors and some common mammalian reservoir hosts. Both species infect the ticks through horizontal transmission from a reservoir host (Bank vole, white-footed mouse) but *B. miyamotoi* can also be transmitted vertically (transovarially) from an infected female tick to her offsprings. (Barbour et al., 2009). *Borrelia miyamotoi* and *B. burgdoferi* have different strategies for their spreading in the same reservoir host and vector species. (Barbour et al., 2009).

According to the knowledge, *B. miyamotoi* DNA was identified for the first time in Europe in bank voles in France (Cosson et al., 2014). Rodents and ticks that were carrying the pathogen came from very diverse environments (forests, hedges bordering grass lands, and also in proximity to human dwellings (Cosson et al., 2014). The symptoms caused by the pathogen *B. miyamotoi* can be confused with symptoms caused by other pathogens like Lyme spirochetes. According to some research, ticks are able to transmit *B.miyamotoi* and Lyme spirochetes at the same time (Cosson et al., 2014). The symptoms caused by the pathogen *B. miyamotoi* include meningoencephalitis in immunocompromised individuals and can also lead to severe disease as well as to co-infection with other pathogens that are also transmitted by *Ixodes* species. A recurrent high density of spirochetemia is characteristic of relapsing fever. Several human cases have been described in Russia, in the USA, in Japan, and the Netherlands (Krause et al., 2015). The relapsing fever group is characterized by influenza-like illness and relapsing episodes of fever and bacteremia. The most common symptoms are febrile illness consisting of high temperatures that can exceed 40 degrees, headache, chills, nausea, and other signs. The patients that were sick due to *B. miyamotoi* experienced several episodes of fever and each relapse was lasting a couple of days.

Two cases were described with a *B. miyamotoi* infection that had an effect on the central nervous system with signs of meningoencephalitis; one patient was an 80-year-old woman from the USA and the other one was a 70-year-old man from the Netherlands. Both diseased had a lymphoproliferative disorder (non-Hodgkin lymphoma and diffuse large B cell lymphoma) and had received chemotherapy before the start of their symptoms (Krause et al., 2015). The geographic distribution of *B. miyamotoi* is not fully known to date (Krause et al., 2015) but according to the above research, it is similar to the Lyme disease distribution. Since the dynamics of transmission of *B. miyamotoi* is not completely understood, it is unknown how often the exposure to the pathogen leads to the infection (Wagemakers et al., 2015). The geographical distribution of *B. miyamotoi* is broader than where the human cases were reported because the pathogen has been found in ticks from different parts of the world (Asia, USA, and Europe). There are also cases of blood transmitted relapsing fever that was caused by *B. recurrentis* and *B. duttonii* whereas regarding *B. miyamotoi* the transmission has only been by experimental study with mouses (Krause et al., 2015). In the Netherlands, it was estimated that each year around 36'000 people are bitten by ticks that were infected with the pathogen *Borrelia miyamotoi*. This can be compared to 183'000 people that are bitten by ticks that carry *B. burgdoferi* (Wagemakers et al., 2015). Causative agents of Lyme borreliosis are only present in

nymphs and adult ticks (Hügli et al., 2009). The spirochetes that cause Lyme borreliosis are rarely transmitted by hard ticks less than 24 hours after the tick's attachment. On the contrary, the spirochetes from the relapsing fever group are transmitted by soft ticks within seconds after the attachment of the ticks because the pathogen is staying in the salivary gland. Scientists haven't yet identified if *B. miyamotoi* is present in the salivary glands or midgut (like the Lyme borreliosis group) and how rapidly it can be transmitted after the attachment of hard ticks. (Wagemakers et al., 2015).

There is another important species vectored by *I. ricinus*: *Anaplasma phagocytophilum*. It is a Gram-negative bacterium that is intracellular (Pilloux et al., 2019). This pathogen infects mainly cells from the bone marrow like neutrophils and granulocytes. It can infect a lot of mammalian hosts. *Anaplasma phagocytophilum* has been found in a really low number in the species *I. ricinus* (Lommano et al., 2012) and there hasn't been any human cases reported in Switzerland (Lommano et al., 2012). *Anaplasma phagocytophilum* is transmitted by the ticks *I. ricinus*. The main symptoms are high fever with myalgia, thrombocytopenia, leucopenia, and depression. In several studies, the pathogen has been detected in Switzerland as well as in Germany, Austria, Scotland, Hungary (Beugnet & Marie, 2009). According to some studies, *A. phagocytophilum* was identified in different rodent species (Beugnet & Marie, 2009) in Switzerland and the UK. Its classic hosts are ruminants and rodents but it has also been found in dogs and cats.

There is also a new bacterium species that has been discovered recently: *Candidatus* Neoehrlichia mikurensis. This Gram negative bacterium has a coccoid form and it belongs to the order Rickettsiales and to the family Anaplasmatacae (Hofmann-Lehmann et al., 2016). Ticks infected with this pathogen were found in Europe, including Switzerland (Oechslin et al., 2017). *Candidatus* Neoehrlichia mikurensis was detected in a dog in Switzerland (Hofmann-Lehmann et al., 2016). Only a limited number of human cases have been reported in Europe, and only one in Switzerland (Hofmann-Lehmann et al., 2016). It is most harmful to patients that have an immune deficiency (Oechslin et al., 2017). *Candidatus* N. mikurensis was also found on wild migrating birds (Elena Lommano et al., 2014). Carnivores, mostly dogs and foxes are exposed to the ticks that carry this pathogen. (Hofmann-Lehmann et al., 2016).

Rickettsia species are bacteria also known to be present in Switzerland (Bernasconi et al., 2002; Lommano et al., 2012). There are two species present in Switzerland: *Rickettia helvetica* and *R. monacensis*; the first one is frequently present in ticks in Switzerland. They

also mention the boutonneuse or Mediterranean spotted fever that is transmitted by *Rh. sanguineus*, but this disease was probably imported to Switzerland, Ticino according to Bernasconi et al. (1997). It is caused by *R. conorrii*. In Switzerland, it was shown in a previous study that *Rickettsia* spp. may associate with birds (Lommano et al., 2014). Different species of birds were found to carry the pathogen *R. helvetica; Turdus merula* (common blackbird), *Anthus* spp. (pipits). According to some research (Lommano et al., 2014), common blackbirds have an important role in the life cycle of *Rickettsia* spp. because they are the carriers of infected ticks. Birds are not the only reservoir host of these bacteria: small mammals are also a potential source of infection (Lommano et al., 2014).

Another significant pathogen transmitted by ticks in Switzerland is *Coxiella brunetii*. *Coxiella brunetii* is a Gram-negative bacterium that causes the disease called Q fever (Pilloux et al., 2019). According to Pilloux et al. (2019), there has been 2 major outbreaks of human cases of the Q fever in the Western part of Switzerland in the years 1983 and 2012. They also state that during these 2 outbreak sheep flocks were probably the source of the disease and that is was transmitted to humans through aerosols, but the potential role played by the ticks in the spreading of the disease during the outbreaks was not studied at the time.

Another important group of pathogens transmitted by ticks in Switzerland are the viruses. Relevant diseases include tick-borne encephalitis or TBE (Gäumann et al., 2010). It is a viral disease that mostly affects humans after the bite of a tick. TBE virus is part of the Flavivirus genus and is an Arbovirus. *Ixodes ricinus* is the main vector of this pathogen. TBE virus can be transmitted transovarially (which is the rarest form of transmission) and transstadially or even between ticks feeding on the same host (Gäumann et al., 2010). Nymphs have a major role in the transmission of the disease because they're more numerous than adult ticks (Rieille et al., 2014). TBE in Switzerland was described for the first time in humans in the year 1969. It is considered a notifiable disease in Switzerland due to the increase in the number of cases (Lommano et al., 2012) with the peak being in 2017 with 257 cases in that year (Casati Pagani et al., 2019). In Switzerland, in the past 10 years, there was an average of 210 cases per year (Casati Pagani et al., 2019). In the Southern part of Switzerland, Ticino, no previous cases of the disease had been reported. The study conducted in 2019 showed for the first time the presence of the pathogen in a non-endemic region (Casati Pagani et al., 2019). The TBE virus has 2 subtypes that are recognized: the Eastern Variant that is most prevalent in the far East of Europe and the Western Variant that is present in the central and occidental Europe. *Ixodes ricinus* is responsible for the spreading of the latter (Beugnet & Marie, 2009). Cases in humans

tend to occur during the peak activity of ticks, mostly during April and November (Beugnet & Marie, 2009). For humans, the most typical clinical signs are non-specific flu-like symptoms followed by asymptomatic intervals and a second stage disease with four clinical manifestations that are the following: meningoencephalitis, meningitis, meningoencephalomyletitis, meningoradiucloneuritis. For dogs, the virus will mostly cause febrile syndrome and encephalitis. The infection can have different levels of manifestations, from lethal to peracute as well as subacute and chronic. In Switzerland to know and estimate the natural foci, the Swiss Federal Office of Public Health uses the registered number of human cases and the detection of the virus in the ticks. A study showed that TBEV is present with 0.46% prevalence (Casati Pagani et al., 2019) in Switzerland. It is in the range of prevalence in Europe that is 0.1-5%. Recent studies showed that the pathogen TBEV is absent in *I. ricinus* that were questing in the urban area of Switzerland (Casati Pagani et al., 2019). Small mammals like small rodents are considered to play an important role in the transmission cycle of the virus but they have a low viral transmission potential to tick because they have a short viraemic phase (Casati Pagani et al., 2019). Tick-borne encephalitis is becoming an increasing health problem in the past years. 2018, according to some research, was the year with the highest number of cases ever seen in Switzerland (Rubel & Brugger, 2020), i.e. 377 cases reported.

Finally, the last group of pathogens that has importance in Switzerland are protozoa, particularly *Babesia* species. Babesiosis is an important disease in Europe but a bit less important and present in Switzerland. According to Lommano et al. (2012), babesiae are only present in a few numbers of ticks in Switzerland. Previously it has been stated that within Switzerland less than 2% of the ticks were infected by babesia (Oechslin et al., 2017). These protozoan parasites will infect the erythrocytes (Tunis, 1981). Babesiosis is one of the most important tick-borne diseases in the world for animals and humans (Casati et al., 2006). Three zoonotic species are involved in Europe: *B. venatorum, B. divergens, B. microti*. *Babesia divergens* is usually a cattle parasite but it is the one that infects most humans in Europe. Humans affected are usually splenectomized patients (Tunis, 1981). Canine babesiosis is considered nowadays an emerging infectious disease in several countries in Europe due to its spreading (Schaarschmidt et al., 2013). *Babesia canis* is the most common babesia transmitted in Europe. *Dermacentor reticulatus* tick is the main vector. The disease caused by *B.canis* can lead to several symptoms; anemia, fever, hemoglobinuria (Schaarschmidt et al., 2013).

3. Materials and Methods

3.1. Sample collection and identification

In 2019-2020 altogether 177 ticks were collected from humans (n=17), dogs (n=23), cats (n=41), deer (n=8), a rabbit and a hedgehog, as well as form the vegetation at 25 locations in three cantons of southern Switzerland (Figure 1). In addition, 62 rodent liver/spleen tissue DNA extracts available from 2005-2006 (Willi et al., 2007) were also analyzed retrospectively. These samples (2 *Arvicola terrestris*, 11 *Apodemus flavicollis*, 27 *Apodemus* sp., 10 *Myodes glareolus*, 3 *Microtus agrestis* and 2 *Microtus arvalis*, as well as 7 *Mus domesticus*) originated from the canton of Grisons, Switzerland. These rodents were specified before necropsy (Hausser, 1995).

The ticks were stored in 70% ethanol, and their species were morphologically identified according to Estrada-Peña et al. (2017). Pictures of representative specimens (selected based on criteria which serve to delineate *I. ricinus* and *I. inopinatus*: Estrada-Peña et al., 2014; Chitimia-Dobler et al., 2018) were made with a VHX-5000 digital microscope (Keyence Co., Osaka, Japan). However, due to difficulties and contradictions in morphological differentiation between females and nymphs of these two species (Figure 2), molecular identification was performed as outlined below. Larvae were identified morphologically according to Estrada-Peña et al. (2014).

3.2. DNA extraction

DNA was extracted from 141 ticks (selected based on their intact state) individually, with the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instruction, including an overnight digestion in tissue lysis buffer and Proteinase-K at 56 °C, as reported by Hornok et al. (2014). An extraction control was also processed in each set of samples.

3.3. Molecular taxonomic analyses of ticks

A 710 bp-long fragment of the cox1 (cytochrome c oxidase subunit I) gene was amplified from *Rh. sanguineus* s.l. and *I. hexagonus* with a conventional PCR modified from Folmer et al. (1994). The primers HCO2198 (forward: 5’ -TAA ACT TCA GGG TGA CCA AAA AAT CA-3’) and LCO1490 (reverse: 5’ -GGT CAA CAA ATC ATA AAG ATA TTG G-3’) were used in a reaction volume of 25 µl, containing 1 U (0.2 µl) HotStarTaq Plus DNA polymerase, 2.5

µl 10x CoralLoad Reaction buffer (including 15 mM $MgCl_2$), 0.5 µl PCR nucleotide Mix (0.2 mM each), 0.5 µl (1 µM final concentration) of each primer, 15.8 µl ddH_2O and 5 µl template DNA. During the amplification, the initial denaturation step at 95 °C for 5 min was followed by 40 cycles of denaturation at 94 °C for 40 s, annealing at 48 °C for 1 min and extension at 72 °C for 1 min. Final extension was performed at 72 °C for 10 min.

For the identification and molecular-phylogenetic analyses of *I. inopinatus* another PCR was used, which amplifies an approx. 460 bp-long fragment of the 16S rRNA gene of Ixodidae (Black and Piesman, 1994). This method is based on the primers 16S+1 (5'- CTG CTC AAT GAT TTT TTA AAT TGC TGT GG-3') and 16S-1 (5'-CCG GTC TGA ACT CAG ATC AAG T-3'). Other reaction components, as well as cycling conditions were the same as above, except for annealing at 51 °C. The reason of performing this test was threefold: (a) whole body DNA extracts were prepared from *Ixodes* sp. ticks in order to screen tick-borne pathogens, but in a preliminary cox1 PCR only host DNA could be amplified from some of them; (b) the 16S rRNA PCR was used to confirm the identity of *I. inopinatus* during its original description (Estrada-Peña et al., 2014) and (c) in later studies (Chitimia-Dobler et al., 2018; Hauck et al., 2019), in the latter without morphological identification and focusing on one particular site of the gene (position 184/185). During the first part of this analysis pools were made of all *Ixodes* samples (containing five DNA extracts in each), and 16S rRNA PCR products of these were sequenced. For individual testing with the same method, 79 samples were selected, including at least 30% of *Ixodes* sp. ticks from the same host species, ticks from co-infestation of the same host, as well as members of pools which had indication of AG bases at position 184/185 in the chromatogram.

3.4. Molecular analysis of *Borrelia miyamotoi* (Spirochaetales: Spirochaetaceae)

An approx. 730 bp-long fragment of the glycero-phospho-diester phospho-diesterase (glpQ) gene of *B. miyamotoi was amplified with t*he primers glpQ-BM-F2 (5'- ATG GGT TCA AAC AAA AAG TCA CC-3') and glpQ-BM-R1 (5'- CCA GGG TCC AAT TCC ATC AGA ATA TTG TGC AAC - 3'). The reaction volume of 25 µl contained 5 µl of extracted DNA, and 20 µl of reaction mixture including 1 unit HotStarTaq Plus DNA polymerase (5U/µl), 200 µM PCR nucleotide mix, 1 µM each primer and 2.5 µl of 10× Coral Load PCR buffer (15 mM $MgCl_2$ included). Touchdown PCR was used for the amplification, during which an initial denaturation step at 95 °C for 5 min was followed by 10 cycles of denaturation at 94 °C for 30 s, annealing at 62 °C for 30 s (annealing temperature was decreased -1°C/cycle) and extension at 72 °C for

1 min. The first 10 cycles were followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 30 s and extension at 72 °C for 1 min. Final extension was performed at 72 °C for 10 min.

3.5. Molecular screening for *Occidentia massiliensis* (Rickettsiales: Rickettsiaceae)

Recently, a new primer pair was designed to match the heat shock chaperonin protein encoding *GroEL* gene of both *Ori. tsutsugamushi* and *Oc. massiliensis*, amplifying an approximately 680-bp-long part of this gene in case of the latter species. The primers Om-groELf1 (5'-AAA AAA GAA ATG TTA GAA GAT ATT GC-3') and Om-groELr2 (5'-GTA CGT ACW ACT TTA GTT GG-3') were used in a reaction volume of 25 µl, which included 5 µl of extracted DNA, and 20 µl of reaction mixture containing 1 unit HotStarTaq Plus DNA polymerase (5U/µl), 200 µM PCR nucleotide mix, 1 µM each primer and 2.5 µl of 10× Coral Load PCR buffer (15 mM $MgCl_2$ included). For amplification, an initial denaturation step at 95 °C for 5 min was followed by 40 cycles of denaturation at 95 °C for 20 s, annealing at 50 °C for 30 s and extension at 72 °C for 1 min. Final extension was performed at 72 °C for 5 min.

3.6. Molecular screening for trypanosomes (Kinetoplastida: Trypanosomatidae)

DNA samples were also screened with a conventional PCR that amplifies an approx. 900-bp-long fragment of the 18S (SSU) rRNA gene of trypanosomes and related kinetoplastids. The primers 609F (forward: 5'-CAC CCG CGG TAA TTC CAG C-3') (da Silva et al., 2004) and 706R (reverse: 5'-CTG AGA CTG TAA CCT CAA-3') (Ramírez et al., 2012) were used in a reaction volume of 25 µl, which included 5 µl of extracted DNA, and 20 µl of reaction mixture containing 0.5 unit HotStarTaq Plus DNA polymerase (5U/µl), 200 µM PCR nucleotide mix, 1 µM each primer and 2.5 µl of 10× Coral Load PCR buffer (15 mM MgCl2 included). For amplification, an initial denaturation step at 95 °C for 5 min was followed by 40 cycles of denaturation at 94 °C for 40 s, annealing at 49 °C for 1.5 min and extension at 72 °C for 1 min. Final extension was performed at 72 °C for 5 min.

3.7. PCR controls, sequencing and phylogenetic analyses

All above pathogen screening PCRs were run with appropriate sequence-verified positive control (DNA from *Ixodes ricinus* nymph M19, from *Africaniella transversale* Amb2 and *Trypanosoma corvi* II., respectively) and negative control (non-template reaction mixture). Extraction controls and negative controls were PCR negative.

Conventional PCR products were subjected to electrophoresis and consequently stained, visualized in 1% standard agarose gel (SeaKem LE Agarose, Lonza Inc.). Purification and sequencing of the PCR products were done by Biomi Ltd. (Gödöllő, Hungary). Obtained sequences were manually edited, then aligned with GenBank sequences by nucleotide BLASTN program (https://blast.ncbi.nlm.nih.gov). Representative sequences will be submitted to GenBank. Sequences from other studies (retrieved from GenBank) were included in the phylogenetic analyses only if they had nearly 100% coverage with sequences from this study. This dataset was resampled 1,000 times to generate bootstrap values. Phylogenetic analyses were conducted with the Maximum Likelihood method by using MEGA version 7.0.

3.8. Statistical analysis

Infection prevalence data according to tick developmental stages were analyzed by Fisher's exact test (number of tick larvae and nymphs *vs* number of adults compared between host species).

3.9. Ethical permission

Ticks used in this study were provided by veterinarians (who removed them during animal care), by hunters (who collected ticks from game animals killed during hunting) and by human subjects on a volunteer basis. Oral consent was obtained to use these ticks for scientific purposes. Rodent samples were used from a previous study (Willi et al., 2007). Thus, no vertebrate animals were killed or restrained for the purpose of tick collection, therefore no ethical permission was needed.

4. Results

4.1. Ticks species and their molecular-phylogenetic analyses

Altogether 177 ticks were collected from cats (n=41), dogs (n=23), humans (n=17), deer (n=8), a rabbit and a hedgehog, as well as from the vegetation at 25 locations in three counties of southern Switzerland (Table 1). Four tick species were identified: *Ixodes ricinus* (n=162), *I. inopinatus* (n=8), *Rhipicephalus sanguineus* s.l. (n=6) and *I. hexagonus* (n=1). *Ixodes ricinus* was found on four host species (cats, dogs, humans, red deer), whereas *I. inopinatus* on two (cat, rabbit) and *I. hexagonus*, *Rh. sanguineus* s.l. on one (hedgehog and dog, respectively). *Ixodes ricinus* was collected in all four seasons (including winter in case of cats only). The adults of this tick species were active year-round, however, the presence of nymphs on any host or the vegetation was associated with the spring and the summer. Its larvae were found only in summer. All three mating males and females of *I. ricinus* were collected in May-June (Table 1).

Considering developmental stages of *I. ricinus*, only one nymph was found on dogs, while as many as four larvae and eight nymphs on cats, but this was a non-significant association (25 adults and 1 immature on dogs vs 61 adults and 12 immatures on cats: $P = 0.17$). However, in contrast to companion animals (dogs, cats) subadult ticks (larvae and nymphs) predominated on humans (14 immatures and 6 adults), which is a highly significant association ($P<0.0001$).

Considering results of molecular taxonomic analyses, two *R. sanguineus* s.l. cox1 gene haplotypes were identified from the same dog, differing in one nucleotide. Comparison (Blast alignment) showed that these samples from southern Switzerland had 99.8%-100% identity (629-630/630 bp) with *R. sanguineus* s.l. from Italy (KX757904). Similarly, *I. hexagonus* collected in southern Switzerland was genetically most closely related to conspecific ticks reported from Italy, meaning 100% (631/631 bp) identity with the corresponding sequence (MG432679).

Molecular analysis of 79 *Ixodes* specimens revealed a high haplotype diversity among *I. ricinus* specimens, i.e. 30 different 16S rRNA sequences (Table 2). Although certain haplotypes appeared to be more frequently found on cats than any other host species (e.g. No. 3 and 13), this was never a statistically significant association. Regarding the country of origin with the closest match in GenBank, most *I. ricinus* 16S rRNA haplotypes were shared between Switzerland and Slovakia (i.e., 16 out of 30), followed by France (12 out of 30) (Table 2). Ticks

from 13 multiple tick-infestations (up to five *I. ricinus* specimens on the same host individual analyzed) showed the presence of different 16S rRNA haplotypes in the majority of cases (10 out of 13).

Ixodes larvae collected from a rabbit were morphologically identified as *I. inopinatus*, because marginal dorsal (md) setae measured approximately four to five times the length of the 5th scutal seta (sc5), in contrast to larvae of *I. ricinus* with a 3:1 ratio of corresponding setae (Figure 3). The species identity for one of these as well as for another female tick from a cat was confirmed as *I. inopinatus* molecularly with the 16S rRNA gene-specific PCR and sequencing. These two ticks had AG bases at position 184/185 in the 16S rRNA gene (similarly to the originally described specimen of *I. inopinatus*: KM211789). Phylogenetically, these two specimens from Switzerland clustered (as a sister group to *I. ricinus*) together with *I. inopinatus* (some still labelled as *I. ricinus* in GenBank) from several north African and European countries, including Tunisia, Spain, Italy, the Netherlands, Germany, Poland, Austria, Latvia and Sweden (Figures 4-5).

4.2. Molecular screening of rare pathogens in ticks and rodents

tick DNA extracts (n=141) and rodent tissue DNA extracts (n=62) were PCR negative for trypanosomes and members of the genera *Occidentia* and *Orientia*.

However, from a rodent (bank vole: *Myodes glareolus*) sample the sequence of *Borrelia miyamotoi* was successfully amplified. This sequence had 100% identity (648/648 bp) with several sequences in GenBank, including strain EU1 (KJ003844). The relevant bank vole originated in eastern Switzerland (Malans, canton Grisons) and was sampled in October, 2005.

In addition, three *I. ricinus* ticks collected in this study were PCR positive for *B. miyamotoi*, amounting to a prevalence of 2.2% in *I. ricinus* (three out of 135 ticks). From all of them the sequence of *B. miyamotoi* was successfully amplified, showing 100% identity with the above rodent-derived sequence. These ticks included two *I. ricinus* females (removed from dogs on two occasions, in June, 2020 in Versoix, canton Geneva), as well as an *I. ricinus* nymph from a cat (sampled in June 2020 in Givrins, canton Vaud). Thus, all three ticks containing the DNA of *B. miyamotoi* originated in the southwestern "corner" of Switzerland.

5. Discussion

While the occurrence of tick-borne bacteria and protozoan parasites are well-documented in Switzerland, the epidemiological situation might change rapidly. This is in part due to natural causes, such as the ongoing climate change which may result in northward spreading of tick species, but also because of human activity, i.e. inadvertent introduction of exotic tick species. Independently from this, tick-borne pathogens might exist in Switzerland, for which samples collected so far in the country were not tested or were found to be negative, in part because their tick-association or European presence has only been recognized recently.

With this in mind, during this study four tick species were collected and analyzed from the points of view of their seasonality, host associations, molecular-phylogenetic comparison and presence of tick-borne pathogens. In the latter part of the study rodent samples were also analyzed retrospectively.

Regarding the seasonality of *I. ricinus* in Switzerland, questing activity generally lasts from February to November (Herrmann and Gern, 2015), and immature stages feed on rodents between February or March to November (Pérez et al., 2012). Finding of *I. ricinus* on cats in December and February in this study extends this seasonality during the winter.

Human infestation with ticks in southern Switzerland was shown here to be associated with immature ticks (particularly nymphs), which can be explained by their smaller size (i.e., more easily overlooked at the beginning of feeding, and noticed later during engorgement).

During the molecular analysis an extremely high diversity of *I. ricinus* (i.e., 30 different 16S rRNA haplotypes) were identified here. In contrast, no small-scale population genetic divergence was found between local *I. ricinus* populations in Switzerland (Delaye et al., 1997). However, on a continental scale marked genetic heterogeneity was discovered, without a geographical pattern/structure (Noureddine et al., 2011). The latter study did not include samples from Switzerland, and based on literature and GenBank data this country appears to lack haplotype comparison of its tick fauna based on mitochondrial markers (e.g. barcoding gene: cox1 or 16S rRNA, as performed here). On the other hand, when populations of *I. ricinus* were analyzed for their relationships within Europe based on phenotype characteristics, specimens from Switzerland almost completely aligned with others from only one country, Slovakia (Estrada-Peña et al., 1996). This was confirmed by the present results, because the highest number of 16S rRNA haplotypes from Switzerland were shared with Slovakia.

From this point of view it is important that *I. ricinus* larvae and nymphs are the most common ticks on birds in Switzerland (Papadopoulos et al., 2002). Frequent association (thus

transportation) of *I. ricinus* by birds in Europe (e.g. Hornok et al., 2014) will obscure differences between its populations, it will also promote the presence of a high genetic diversity (mixture) on the individual level.

On the other hand, *Rh. saguineus* and *I. hexagonus* are rarely transported by birds (Papadopoulos et al., 2002), therefore a higher rate of geographical structuring (gene flow reduced on a continental level) can be anticipated between their populations. The occurrence of these two tick species in southern Switzerland is well-documented (Bernasconi et al., 1997; 2002), but molecular taxonomic analyses of these two species have not been reported. Based on ticks collected during the present study, comparisons of the mitochondrial cox1 gene showed that *R. sanguineus* and *I. hexagonus* in southern Switzerland are genetically most closely related to conspecific ticks reported from Italy. This "haplotype confluence" of their populations between Switzerland and Italy, or the mid-western Mediterranean, might reflect low rate ground-bound dispersal events.

Ixodes inopinatus (described in 2014 by Estrada-Peña et al.) was originally thought to be a Mediterranean tick species, but later is was also discovered in other parts of Europe (e.g. Hauck et al., 2019). Prior to that, genetic analysis of microsatellites of *I. ricinus* ticks collected in Switzerland showed absence of differentiation at the local scale (even between specimens separated by the Alps), but marked difference between Switzerland and Tunisia (de Meeûs et al. 2002). Later on, *I. inopinatus* was separated as a new species based (in part) on samples collected in Tunisia. However, until now it has not been reported from Switzerland.

Based on the present results, *I. inopinatus* appears to be a rare tick species in southern Switzerland (since it was found only on two occasions, i.e. the rabbit most likely acquired its multiple infestation by contacting a batch of newly hatched larvae). The two molecularly identified specimens had AG bases at position 184/185 in the 16S rRNA gene (similarly to the originally described specimen of *I. inopinatus*: KM211789), fulfilling the criterion suggested by Hauck et al. (2019). At the same time, 16S rRNA gene sequence data from this as well as other studies and GenBank indicate the pan-European presence *of I. inopinatus* (including regions in Scandinavia, Baltic and Benelux countries, as well as central Europe (Poland) and southern Europe (Italy) where it was reported as *I. ricinus*). The cat and the rabbit are new host records for *I. inopinatus*.

All tick DNA extracts and rodent tissue DNA extracts were PCR negative for trypanosomes and *Occidentia*/*Orientia* spp. There are literature data and observations which justify the search for these microorganisms, usually not considered among ixodid tick-borne pathogens. *Occidentia massiliensis* (originally isolated and described from soft ticks:

Mediannikov et al., 2014) has recently been found in hard ticks (Hornok et al., unpublished data). *Orientia tsutsugamushi* (another, mite-borne member of Rickettsiaceae) has been detected in wild living rodents in France, i.e. the country neighboring Switzerland (Cosson et al., 2015). In addition, a novel *Trypanosoma* has recently been described in *I. ricinus* collected in Slovakia (Luu et al., 2020).

Borrelia miyamotoi is a relapsing fever group spirochaete, associated with species of the *I. ricinus* complex and potentially causing periodic febrile illness, particularly in immunocompromised patients. This a zoonotic relapsing fever spirochete, transmitted from rodent/bird reservoirs to humans via the bite of hard ticks (in Europe mainly by *I. ricinus*), in which is also maintained transovarially (unlike Lyme disease spirochaetes) (Siński et al., 2016). The first finding of *B. miyamotoi* in Europe dates back to 1999, when it has been detected in *I. ricinus* ticks in Sweden (Fraenkel et al., 2002).

The prevalence of *B. miyamotoi* in *I. ricinus* collected in Switzerland was reported to be 1% to 2.5 % to in other studies (Lommano et al., 2012; Oechslin et al., 2017), which corresponds to the present finding (2.2%). Interestingly, the latter study reported the highest prevalence of tick-borne *B. miyamotoi* in western Switzerland (Basel, Neuchâtel), which coincides with the geographical aspects of the present findings, i.e. PCR positivity of ticks was detected only in the western part of southern Switzerland. While in the present study *B. miyamotoi*-infected ticks were removed from two dogs and a cat, these pet animals are only known to be susceptible to soft tick-borne relapsing fever spirochaetes (Elelu, 2018).

In a previous study carried out in Switzerland on *B. miyamotoi* in rodents (including the bank vole), blood samples were negative for this spirochaete (Burri et al., 2014).

The bank vole (*My. glareolus*) was shown to be a competent reservoir of *B. miyamotoi*, with the ability to cause infection of tick larvae feeding on them (Burri et al., 2014). Natural infection of the bank vole with *B.miyamotoi* was also reported in Europe (Cosson et al., 2014). However, to the best of our knowledge, this is the first finding of *B. miyamotoi* in a rodent in Switzerland, and (taking into account the year of collection: 2005) in a chronological order this might be the first indication of *B. miyamotoi* in any rodent species in Europe.

6. Summary

English Abstract

In Europe, ticks are among the most important transmitters (vectors) of pathogens causing diseases in domestic or wild animals and humans. While the occurrence of tick-borne bacteria and protozoan parasites are well-documented in Switzerland, the epidemiological situation might change rapidly. This is in part due to natural causes, such as the ongoing climate change which may result in northward spreading of tick species, but also because of human activity, i.e. inadvertent introduction of exotic tick species. The aim of our study was to collect ticks from various host species in Switzerland, to identify the tick species morphologically and molecularly, as well as to screen rare, hitherto not reported tick-borne pathogens in these samples. We also included rodent tissues in these analyses.

In 2019-2020 altogether 177 ticks were collected from the vegetation, as well as from humans (n=17), dogs (n=23), cats (n=41), deer (n=8), a rabbit and a hedgehog at 25 locations in three counties of southern Switzerland. Sixty two rodent liver/spleen tissue DNA extracts (representing three species and further two genera) available from 2006 were also analysed retrospectively. Four tick species were identified: *Ixodes ricinus* (n=162), *I. inopinatus* (n=8), *Rhipicephalus sanguineus* (n=6) and *I. hexagonus* (n=1). In contrast to companion animals (dogs, cats) subadult ticks (larvae and nymphs) predominated on humans, which is a highly significant association ($P<0.0001$). Molecular taxonomic analysis showed that *R. sanguineus* and *I. hexagonus* in southern Switzerland is genetically most closely related to conspecific ticks reported from Italy. All tick DNA extracts (n=141) and rodent tissue DNA extracts (n=62) were PCR negative for trypanosomes (recently reported in *I. ricinus* in Slovakia) and *Occidentia*/*Orientia* spp. (known to occur in ticks/rodents). However, from a rodent (*Myodes glareolus*) sample the sequence of *Borrelia miyamotoi* was succesfully amplified. This a zoonotic relapsing fever spirochete, transmitted from rodent/bird reservoirs to humans via the bite of hard ticks (in Europe mainly by *I. ricinus*). To the best of our knowledge, this is the first finding of *I. inopinatus* in Switzerland, and of *B. miyamotoi* in a rodent in Switzerland. Considering the latter (taking into account the year of collection: 2005) in a chronological order this might be the first indication of *B. miyamotoi* in any rodent species in Europe.

Hungarian Abstract

Európában a kullancsokat a házi vagy vadon élő állatokat és az embert megbetegítő kórokozók legfontosabb terjesztői (vektorai) között tartják számon. Noha a kullancs közvetítette baktériumok és egysejtű élősködők előfordulása Svájcban jól dokumentált, ez a járványtani helyzet gyorsan változhat. Ez részben természetes okoknak tulajdonítható, amilyen a jelenleg zajló klímaváltozás (amely a kullancsfajok észak felé való terjedését eredményezhzeti), de emberi tevékenység is kiválthatja, például egzotikus kullancsfajok véletlen behurcolásával. Vizsgálatunk célja az volt, hogy kullancsokat gyűjtsünk Svájcban különféle gazdafajokról, a kullancsok faját morfológiai és molekuláris alapon meghatározzuk, és egyúttal ritka, az országban eddig nem jelentett kórokozókat keressünk bennük. Ez utóbbi tesztekben rágcsáló mintákat is felhasználtunk.

2019-2020 folyamán 177 kullancsot gyűjtöttünk Svájc déli részén három megyében összesen 25 helyen: a növényzetről, emberről (17 fő), kutyákról (23 egyed), macskákról (41 egyed), szarvasról (nyolc egyed), valamint egy-egy nyúlról és sünről. 2006-ban gyűjtött 62 rágcsáló (amelyek három fajt és további két nemet képviseltek) máj/lép szövetéből kivont DNS-t is elemeztünk retrospektíve. Négy kullancsfajt azonosítottunk: *Ixodes ricinus* (n=162), *I. inopinatus* (n=8), *Rhipicephalus sanguineus* (n=6) and *I. hexagonus* (n=1). A társállatokkal (kutyák, macskák) szemben emberen többségben voltak a kullancslárvák és -nimfák, ami egy erősen szignifikáns összefüggés ($P<0.0001$). Molekuláris rendszertani vizsgálatuk alapján a *R. sanguineus* és az *I. hexagonus* Svájc déli részén genetikailag az azonos fajú, olaszországi kullancsokhoz állnak legközelebb. Minden kullancs (n=141) és rágcsáló (n=62) DNS minta PCR negatív volt trypanosomákra (ezeket nemrég leírtak Szlovákiában *I. ricinus* fajból) és *Occidentia*/*Orientia*-fajokra (amelyek előfordulása ismert kullancsokban, ill. rágcsálókban). Azonban egy rágcsáló (*Myodes glareolus*) mintából sikerült felerősíteni a *Borrelia miyamotoi* szekvenciáját. Ez egy zoonótikus, visszatérő lázat okozó spirokéta, amely rágcsáló/madár gazdából kullancs csípéssel terjedhet emberre (Európában főleg *I. ricinus* által). Legjobb tudomásunk szerint ez az első azonosítása az *I. inopinatus* kullancsfajnak Svájcban, és a *B. miyamotoi* fajé svájci rágcsálóban. Az utóbbit tekintve és figyelembe véve a gyűjtés évét (2005) időrendi sorrendben valószínűleg a rágcsálók fertőzöttségének első bizonyítéka Európában.

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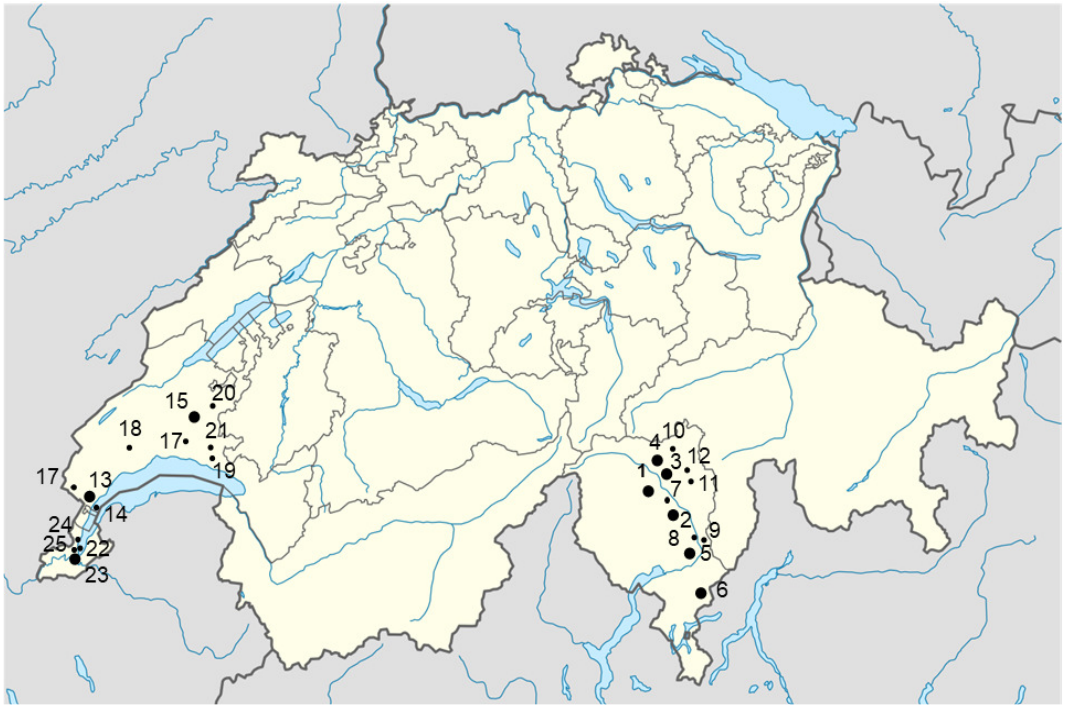
Figure 1: map with collection site



1. Chironico
2. Biasca
3. Malvaglia
4. Dongio
5. Bellinzona
6. Val Colla
7. Pollegio
8. Claro
9. Lumino
10. Castro
11. Marolta
12. olivone

Ticino county

13. Givrins
14. Nyon
15. Martherenges
16. Les tavernes
17. St-Cergue
18. Le bois de bussy
19. Bois du jorat
20. St Cierges
21. Cornier

Vaud county

22. Versoix
23. Geneva
24. Collex
25. Bellvue

Geneva county

Figure 2: Pictures of representative specimens *I. ricinus* and *I. inopinatus*

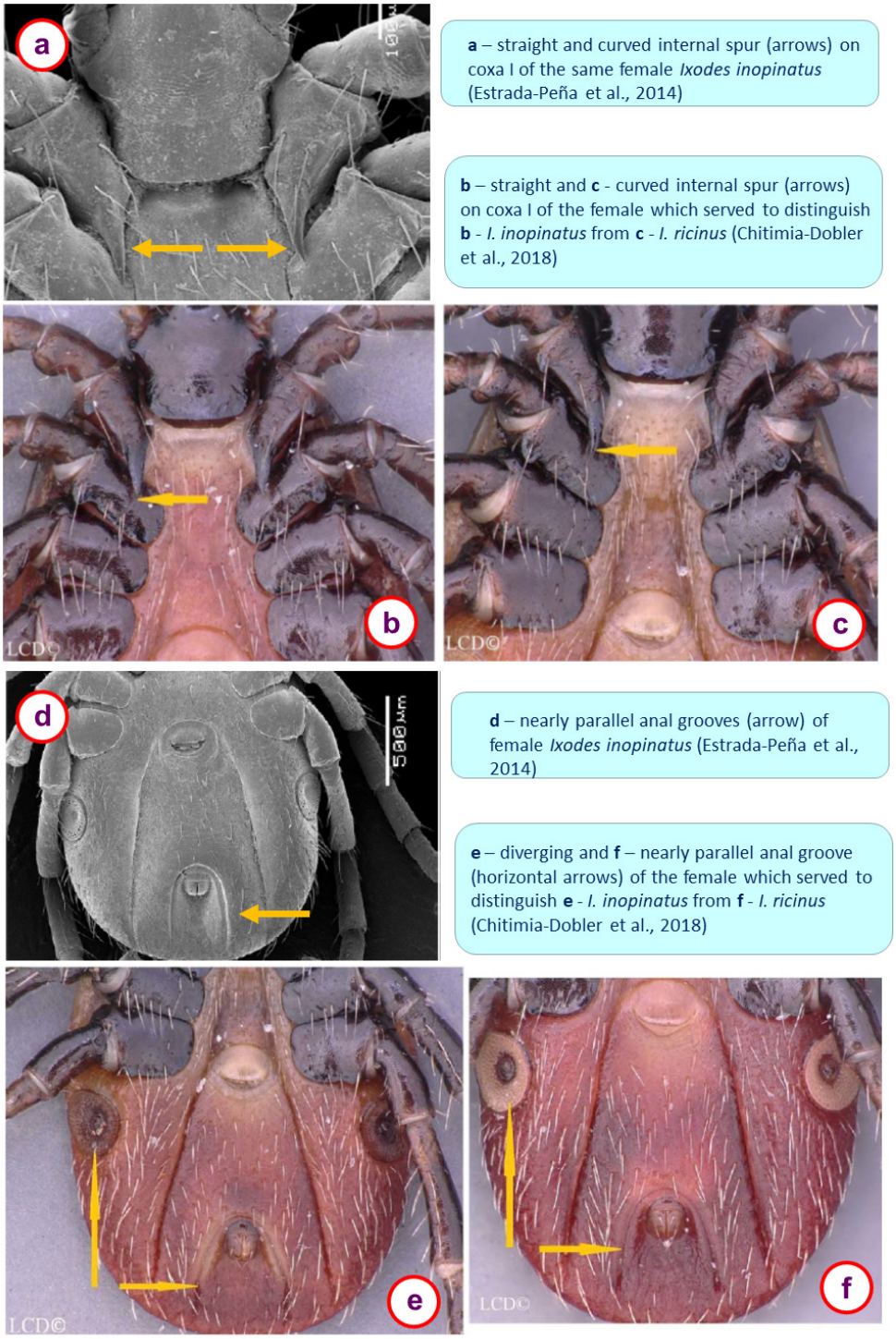


Figure 3: Pictures of representative specimens *I. ricinus* and *I. inopinatus*

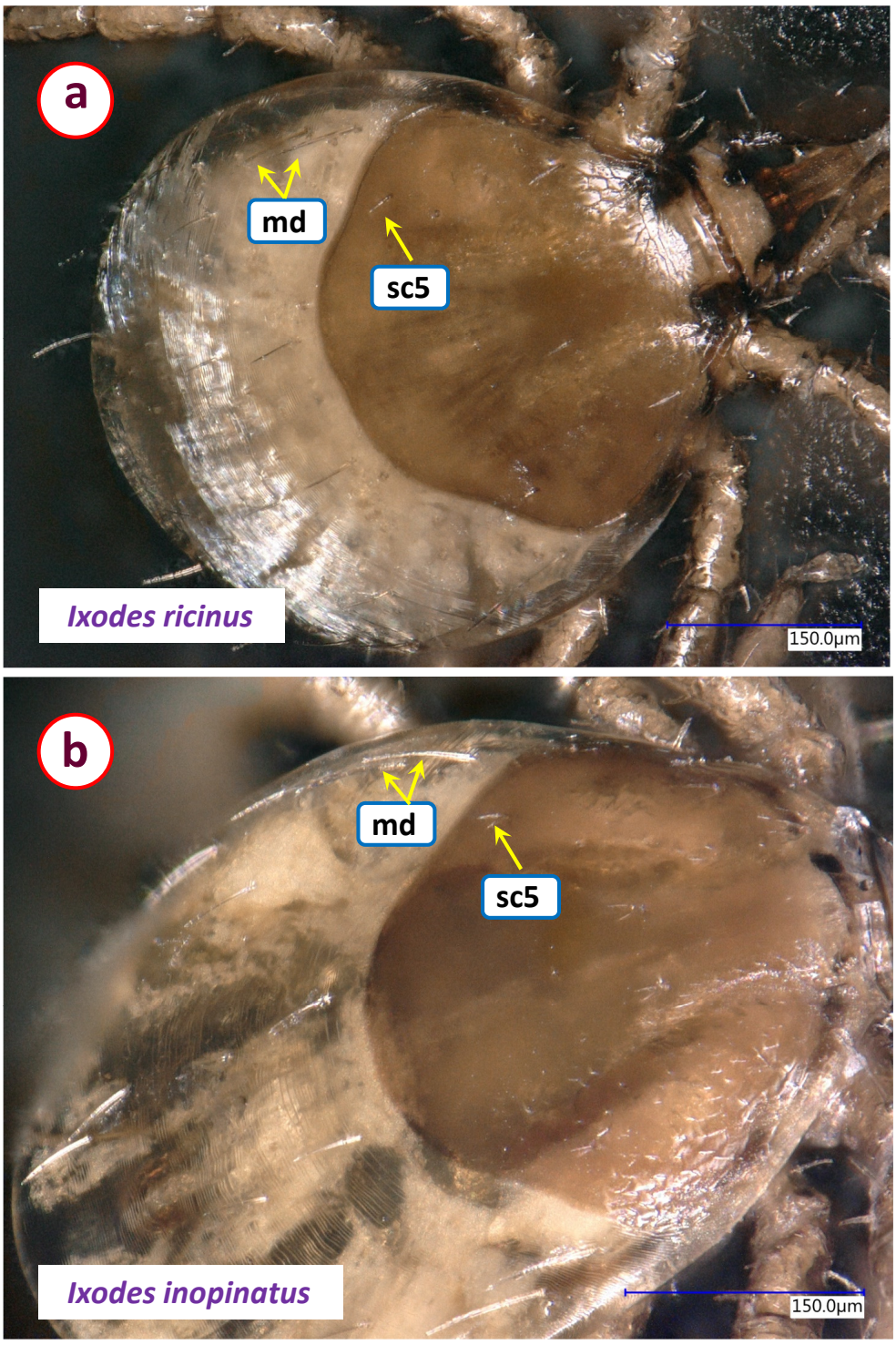
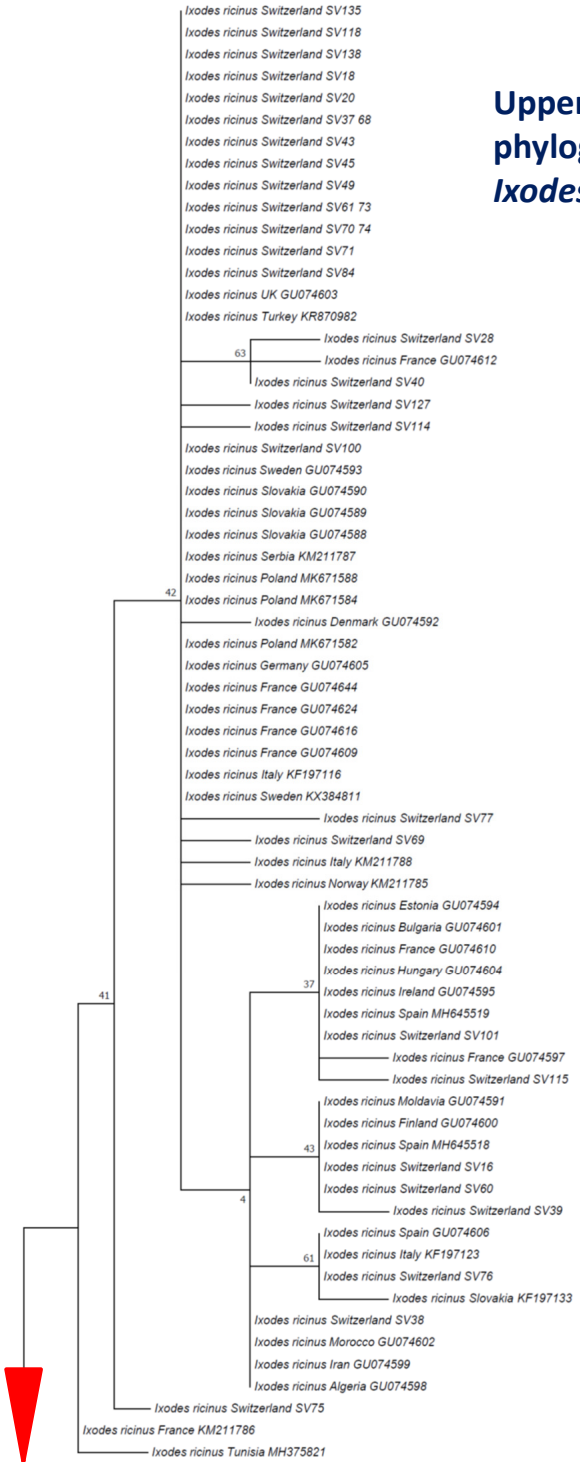


Figure 4



Upper part of the phylogenetic tree: *Ixodes ricinus* group

Figure 5

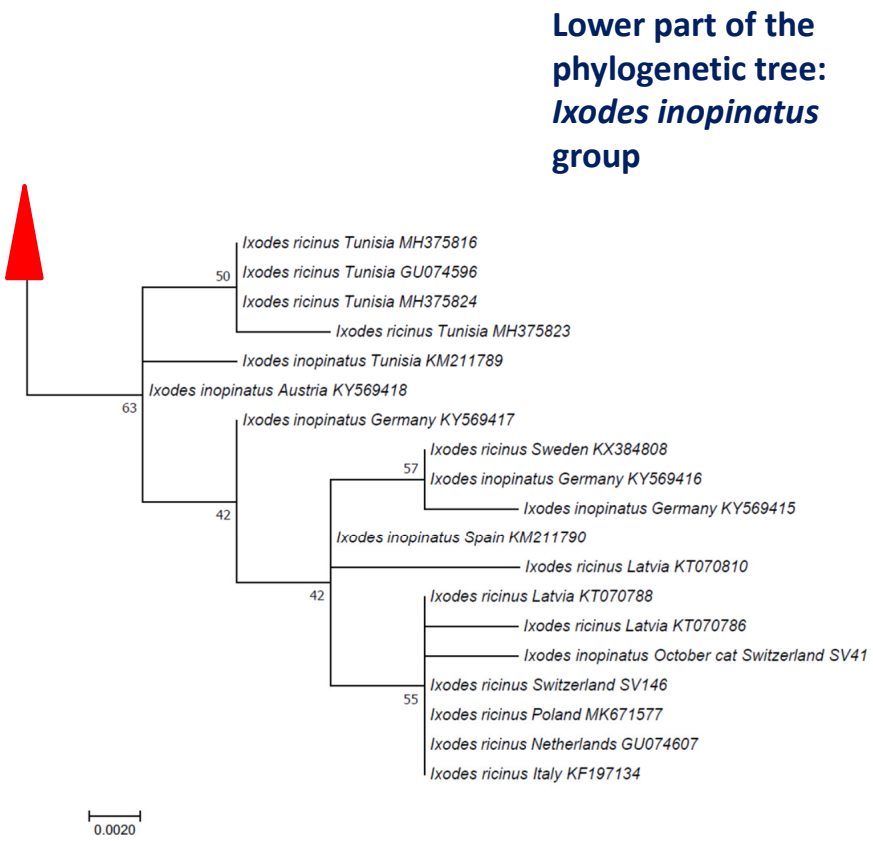

Lower part of the phylogenetic tree: *Ixodes inopinatus* group
Ixodes ricinus Tunisia MH375816
Ixodes ricinus Tunisia GU074596
Ixodes ricinus Tunisia MH375824
Ixodes ricinus Tunisia MH375823
Ixodes inopinatus Tunisia KM211789
Ixodes inopinatus Austria KY569418
Ixodes inopinatus Germany KY569417
Ixodes ricinus Sweden KX384808
Ixodes inopinatus Germany KY569416
Ixodes inopinatus Germany KY569415
Ixodes inopinatus Spain KM211790
Ixodes ricinus Latvia KT070810
Ixodes ricinus Latvia KT070788
Ixodes ricinus Latvia KT070786
Ixodes inopinatus October cat Switzerland SV41
Ixodes ricinus Switzerland SV146
Ixodes ricinus Poland MK671577
Ixodes ricinus Netherlands GU074607
Ixodes ricinus Italy KF197134
50
63
42
57
42
55
0.0020

Table 1. Hosts, species and stages of ticks from Switzerland (2019-2020) according to month of collection. The DNA was extracted from 141 out of 177 ticks, individually. The species identified for the first time in Switzerland is highlighted with red, bold fonts.

Origin		Tick					
Generic name	Number	Species	Total number	Stage or sex (month of collection)			
				Female	Male	Nymph	Larva
Human	17	*Ixodes ricinus*	20	2 (June), 4 (July)	-	4 (May), 6 (June), 2 (July)	2 (Aug.)
Dog	23	*Rhipicephalus sanguineus*	6	-	-	6 (Aug.)	-
		Ixodes ricinus	26	❶	1 (April)	1 (June)	-
Cat	41	***Ixodes inopinatus***	1	1 (October)	-	-	-
		Ixodes ricinus	73	❷	2 (Feb.), 2 (May) ⚥, 2 (June) ⚥⚥	1 (March), 1 (April), 1 (May), 2 (June), 3 (Aug.)	4 (June)
Red deer	8	*Ixodes ricinus*	29	1 (June), 3 (July), 17 (Sept.)	-	-	-
Rabbit	1	***Ixodes inopinatus***	7	-	-	-	7 (Aug.)
Hedgehog	1	*Ixodes hexagonus*	1	1 (June)	-	-	-
Vegetation	4 occasions	*Ixodes ricinus*	14	1 (July)	-	1 (June)	12 (July)

Marks: ⚥ Mating with a female
❶ 1 (April), 7 (May), 6 (June), 3 (July), 2 (August), 3 (September), 1 (October), 1 (November)
❷ 5 (Feb.), 4 (March), 10 (April), 14 (May), 10 (June), 7 (August), 1 (September), 2 (October), 2 (December)

Abbreviations: Feb. - February, Aug. - August, Sept. - September

Table 2. 16S rRNA haplotypes of *Ixodes inopinatus* (Nos. 1-2.) and *I. ricinus* (Nos. 3-30) according to their geographical relevance (country, based on closest GenBank matches), number of hosts (origin).

No.	Country (closest GenBank match)	Origin					
		cat	dog	human	deer	rabbit	veg.
1.	Netherlands (GU074607), Tunisia (GU074596)	1	-	-	-	-	-
2.	Netherlands (GU074607), Italy (KF197134)	-	-	-	-	1	-
3.	Slovakia (GU074590)	5	-	2	1	-	1
4.	Slovakia (GU074589), France (GU074610), Morocco (GU074602)*, Turkey (KR870982), Algeria (GU074598)*	1	-	-	-	-	-
5.	Slovakia (GU074589), Turkey (KR870982)	1+	-	-	-	-	-
6.	France (GU074644)	-	1	-	-	-	-
7.	Finland (GU074600), Algeria (GU074598)*, Spain (MH645518), Moldova (GU074591)	-	-	1	-	-	-
8.	Slovakia (GU074589), Turkey (KR870982)	3	2	5	3	-	-
9.	Slovakia (GU074589), Turkey (KR870982)	1	-	-	-	-	-
10.	Slovakia (GU074588, GU074589), Turkey (KR870982)	-	2	-	1	-	-
11.	Slovakia (GU074588)	-	1	1	-	-	-
12.	Slovakia (GU074590)	1	-	-	-	-	-
13.	Spain (GU074606, MH645522)	5	1	4	2	-	-
14.	Slovakia (GU074589), Turkey (KR870982)	1	-	-	-	-	-
15.	France (GU074624, GU074630)	1	-	1	-	-	-
16.	Ireland (GU074595), Spain (MH645519)	-	1	2	2	-	4
17.	Slovakia (GU074589), Turkey (KR870982)	-	-	1	-	-	-
18.	Spain (MH645521), France (GU074616)	-	-	1	2	-	-
19.	France (GU074644)	-	1	1	1	-	-
20.	France (GU074645, GU074610), Slovakia (GU074589), Morocco (GU074602)*, Finland (GU074600), Algeria (GU074598)*, Spain (MH645518), Turkey (KR870982), Moldova (GU074591)	-	-	-	1	-	-
21.	Spain (MH645521), France (GU074645, GU074616), Algeria (GU074598)*, Estonia (GU074594)	-	-	-	1	-	-
22.	Spain (MH645521), France (GU074644, GU074616), Iran (GU074599)	-	-	-	1	-	-
23.	France (GU074609)	1	-	-	-	-	-
24.	Spain (MH645521), France (GU074616, GU074630)	-	1	-	-	-	-
25.	France (GU074610), Slovakia (GU074589), Turkey (KR870982)	-	-	1	-	-	-
26.	Slovakia (GU074588, GU074589), Turkey (KR870982)	1	-	-	-	-	-
27.	Slovakia (GU074589, GU074590), Spain (MH645521), Turkey (KR870982), France (GU074616)	1	-	-	-	-	-
28.	France (GU074612)	-	-	-	1	-	-
29.	Sweden (GU074593), Slovakia (GU074588)	-	2	-	-	-	-
30.	Slovakia (GU074588)	1	-	-	-	-	-

Marks: * Mistakenly labelled as *Ixodes inopinatus* in GenBank (October 14, 2020). + Host unknown

Abbreviation: veg. - vegetation

1