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Further thoughts on the taxonomy and vector role of *Rhipicephalus sanguineus* group ticks

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ABSTRACT

Rhipicephalus sanguineus is a tick species described in 1806 by Latreille, based on specimens probably collected in France. However, this is a taxon with uncertain morphological definition and recent studies have gathered irrefutable evidence supporting the existence of a cryptic species-complex under the name '*R. sanguineus*', whose number of sibling species around the world has yet to be ascertained. This fact is of great medical and veterinary concern, also considering that ticks currently identified as '*R. sanguineus*' have been regarded as proven or putative vectors of several pathogens infecting dogs and humans. Differences in the distribution and prevalence of some of these microorganisms (e.g., *Ehrlichia canis* and *Hepatozoon canis*) further support the existence of distinct species under the name '*R. sanguineus*' and suggest that the vector competence of these tick species may vary. This article provides an account on the taxonomy and the vector role of ticks belonging to the *R. sanguineus* group in the light of recent research.

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1. Introduction

Rhipicephalus sanguineus sensu stricto (s.s.) was originally described in 1806 as *Ixodes sanguineus* (Latreille, 1806). While its description was accepted for that time, when tick taxonomy was still in its infancy, it was a rather poor, incomplete description, as follows: "*Sanguineus, punctatus, postice lineolis tribus impressus; dorso antico macula nulla thoracica, distincta*" (Latreille, 1806); that is, "blood red, punctate, posteriorly with three impressed lines; no distinct 'thoracic' spot anterodorsally". Furthermore, Latreille provided almost no detail regarding the type specimen of the so-called '*I. sanguineus*', being the only

information provided "*habitat in Gallia*" (Latreille, 1806). Indeed, *Gallia* is the Latin name given by the Romans to the territories where the Celtic Gauls lived in the Iron Age and the Roman period (*circa* from the 5th century BC to the 3rd century AD). As a matter of fact, *Gallia* included present-day France, Belgium, Luxemburg and parts of the Netherlands, Switzerland, Germany on the west bank of the Rhine, and the Po Valley (Arrowsmith, 1832). Nonetheless, most tick taxonomists agree that the type locality of *R. sanguineus* s.s. is in France (Nava et al., 2012).

Subsequently, several renowned tick taxonomists (N. A. Filippova, H. Hoogstraal, I. G. Horak, C. L. Koch, P. C. Morel, L. G. Neumann, J. B. Walker and F. Zumpt, to name but a few) endeavoured to produce reliable keys and/or morphological accounts for *R. sanguineus* s.s. and related species (e.g., Koch, 1844; Neumann, 1911; Morel and Vassiliades, 1963; Filippova, 1997; Walker et al., 2000). The most recent re-description available for this species may be found in "The genus *Rhipicephalus* (Acari, Ixodidae): a guide to the brown

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ticks of the world”, a reference textbook for *Rhipicephalus* ticks (Walker et al., 2000). Nonetheless, these morphological accounts are not reliable because they were based on specimens collected in different continents and may actually represent different species.

The name '*R. sanguineus*' is supported by its usage and it was considered as a valid species in several world lists of tick names (Camicas et al., 1998; Horak et al., 2002; Barker and Murrell, 2004; Guglielmone et al., 2010, 2014; Guglielmone and Nava, 2014). However, results from several crossbreeding and genetic studies (Bernasconi et al., 2002; Szabó et al., 2005; Burlini et al., 2010; Moraes-Filho et al., 2011; Levin et al., 2012; Nava et al., 2012; Liu et al., 2013; Dantas-Torres et al., 2013) suggested that ticks currently identified as '*R. sanguineus*' actually include a group of sibling species. Indeed, the most recent world list of tick names (Guglielmone et al., 2014) considered that besides *Gallia* (type locality), records of *R. sanguineus* s.s. from around the world are currently considered speculative, as they represent a yet unknown number of valid species (*R. sanguineus* sensu lato). Nonetheless, even ticks collected from dogs in France should be at present referred to as *R. sanguineus* s.l., since genetically distinct populations of ticks have been reported to occur in this country (Dantas-Torres et al., 2013).

In recent years, several aspects related to the taxonomy, biology, ecology, and vector role of *R. sanguineus* s.l. have been reviewed in the international literature (Dantas-Torres, 2008, 2010; Gray et al., 2013). This article provides an account on the taxonomy and the vector role of ticks belonging to the *R. sanguineus* group in the light of recent research.

2. The *Rhipicephalus sanguineus* group

For quite some time, the *R. sanguineus* group has been subject to considerable debate among taxonomists from around the world (Zahler et al., 1997). Camicas et al. (1998) listed 17 species for this group (Table 1).

While some ticks belonging to this group are fairly different from a morphological perspective, some of them are similar and difficult to identify on morphological grounds alone. For instance, *R. pusillus* is very distinct morphologically and also genetically from some species belonging to the *R. sanguineus* group (Zahler et al., 1997). Indeed, a more profound assessment of its affiliation to the *R. sanguineus* group has been encouraged (Zahler et al., 1997). As another example, *R. rossicus* is quite similar morphologically, and genetically very close, to *R. pumilio* and it has been suggested that they may be conspecific (Zahler et al., 1997) or, perhaps, subspecies (Beati and Keirans, 2001).

The core problem of this group is perhaps the absence of a morphological definition for *R. sanguineus* s.s. In particular, this tick has long been confounded with *R. turanicus* (and vice versa). Pegram et al. (1987) attempted to clarify the status of this species, but it is uncertain whether *bona fide* *R. turanicus* specimens were involved in their work, as emphasized by Guglielmone and Nava (2014). Later on, Filippova (1997) re-described *R. turanicus* and provided a *bona fide* morphological definition for the species. Genetic data for *R. turanicus* collected by flagging in Turkmenistan

Table 1

Species of the *Rhipicephalus sanguineus* group (sensu Camicas et al., 1998).

Species and authorities	Geographical distribution
<i>Rhipicephalus aurantiacus</i> (Neumann, 1907)	Afro-tropical
<i>Rhipicephalus bergeoni</i> (Morel and Balis, 1976)	Afro-tropical
<i>Rhipicephalus boueti</i> (Morel, 1957)	Afro-tropical
<i>Rhipicephalus camicasi</i> (Morel, Mouchet and Rodhain, 1976)	Afro-tropical, Palearctic
<i>Rhipicephalus guilhoni</i> (Morel and Vassiliades, 1963)	Afro-tropical
<i>Rhipicephalus leporis</i> (Pomerantzev, 1946)	Palearctic
<i>Rhipicephalus moucheti</i> (Morel, 1965)	Afro-tropical
<i>Rhipicephalus pumilio</i> (Schulze, 1935)	Palearctic
<i>Rhipicephalus pusillus</i> (Gil Collado, 1936)	Palearctic
<i>Rhipicephalus ramachandrai</i> (Dhanda, 1966)	Oriental
<i>Rhipicephalus rossicus</i> (Yakimov and Kol-Yakimova, 1911)	Palearctic
<i>Rhipicephalus sanguineus</i> (Latreille, 1806)	Cosmopolitan ^a
<i>Rhipicephalus schulzei</i> (Olenev, 1929)	Palearctic
<i>Rhipicephalus sulcatus</i> (Neumann, 1908)	Afro-tropical
<i>Rhipicephalus tetracornus</i> (Kitaoka and Suzuki, 1983)	Oriental
<i>Rhipicephalus turanicus</i> (Pomerantzev, 1940)	Afro-tropical, Oriental, Palearctic
<i>Rhipicephalus ziemanni</i> (Neumann, 1904)	Afro-tropical

^a *R. sanguineus* has long been considered to be a cosmopolitan tick species, but this is a taxon with uncertain morphological definition and, therefore, its geographical distribution is uncertain as well.

and identified by Filippova is now available in GenBank (KF145150.1 for 16S, KF145151.1 for 12S and KF145152.1 and KF145153.1 for *cox1*) (Dantas-Torres et al., 2013). However, in the absence of a morphological definition and specific genetic markers for *R. sanguineus* s.s., its precise discrimination with *R. turanicus* is still a difficult task.

In addition to the lack of a morphological definition for *R. sanguineus* s.s., recent genetic studies assessing the variability of ticks collected from dogs in different continents were consistent in demonstrating the existence of two well distinct populations under the name '*R. sanguineus*' (Szabó et al., 2005; Burlini et al., 2010; Moraes-Filho et al., 2011; Levin et al., 2012; Nava et al., 2012; Dantas-Torres et al., 2013; Liu et al., 2013). For example, an analysis of mitochondrial 16S rDNA sequences from *R. sanguineus* s.l. collected in 29 localities of nine Latin American countries with those from South Africa, Spain, and Italy revealed the existence of two main phylogenetic clades and six different haplotypes (Moraes-Filho et al., 2011). One clade included three haplotypes (A–C) from Latin America related to ticks from South Africa, which were all designated as the 'tropical' species. The other group included the three remaining haplotypes (D–F) from Latin America, which were related to ticks from Europe and designated as the 'temperate' species. As a corollary, another investigation also suggested the existence of two main groups, one including haplotypes from localities of Argentina, Uruguay and Chile (namely, the 'Southern' lineage) and other clustering haplotypes from Brazil, Paraguay, Colombia, South Africa, Mozambique, and from two localities of Northern Argentina (the 'Northern' lineage) (Nava et al., 2012). The authors concluded that ticks from the Northern lineage and from Mozambique and South Africa represent a single species that is not *R. sanguineus* s.s.,

Table 2
Pathogens transmitted by *Rhipicephalus sanguineus* sensu lato to dogs and humans (Dantas-Torres, 2008; Dantas-Torres et al., 2012b).

Pathogen	Disease name	Zoonotic potential
<i>Acanthocheilonema dracunculoides</i>	Subcutaneous filariasis	No
<i>Anaplasma platys</i>	Infectious canine cyclic thrombocytopenia	Yes
<i>Babesia gibsoni</i>	Babesiosis	No
<i>Babesia vogeli</i>	Babesiosis	No
<i>Cercopithifilaria bairdii</i>	Subcutaneous filariasis	No
<i>Cercopithifilaria grassii</i>	Subcutaneous filariasis	No
<i>Coxiella burnetii</i>	Q fever	Yes
<i>Ehrlichia canis</i>	Monocytic ehrlichiosis	Yes
<i>Hepatozoon canis</i>	Hepatozoonosis	No
<i>Leishmania infantum</i>	Visceral leishmaniosis	Yes
<i>Mycoplasma haemocanis</i>	Haemobartonellosis	No
<i>Rickettsia conorii</i>	Mediterranean spotted fever (Boutonneuse fever)	Yes
<i>Rickettsia massiliae</i>	Spotted-fever	Yes
<i>Rickettsia rickettsii</i>	Rocky Mountain spotted fever	Yes

whereas ticks from the Southern lineage and from Western Europe probably represent the true *R. sanguineus* s.s.

Additional research has shed more light on the matter. We examined ticks collected in 17 countries and assessed their genetic variability using three genetic markers (i.e., 16S rDNA, 12S rDNA, and *cox1*). Results from this study showed that in addition to the two previously characterized lineages of *R. sanguineus* s.l., dogs may be infested by other tick species, including *R. turanicus* and two morphologically and genetically distinct lineages of ticks designated as *Rhipicephalus* sp. I and *Rhipicephalus* sp. III (Dantas-Torres et al., 2013). The so-called tropical species (=northern lineage) is present in all continents and it forms a sister group with *R. guilhoni*. Finally, a more recent study also revealed that *R. rossicus* is the predominant species found on dogs in the steppe region of south-eastern Romania (Dumitrache et al., 2014). Altogether, these studies indicate that the situation in the Old World is much more complicated than in the New World, where tropical and temperate lineages of *R. sanguineus* s.l. are apparently the only representatives of the *R. sanguineus* group found on dogs.

3. *Rhipicephalus sanguineus* group and associated pathogens

With some exceptions, there is little information regarding the vector role of ticks belonging to the *R. sanguineus* group. Ticks identified as *R. sanguineus* s.l. have been regarded as proven or putative vectors for a number of pathogenic microorganisms, including bacteria, protozoa, and nematodes (Table 2). Most of these pathogens have been implicated as causative agents of diseases in dogs, but some of them may also infect humans (reviewed in Dantas-Torres et al., 2012b).

Studies carried out in different parts of the world suggest that there might be differences in terms of vector competence among populations (or sibling species) of *R.*

sanguineus s.l. In October 2009, an outbreak of canine hepatozoonosis was reported in a confined dog population in southern Italy and a high prevalence of *Hepatozoon canis* infection was recorded in engorged nymphs of *Rhipicephalus* sp. I collected from the immediate environment where the dogs lived (Dantas-Torres et al., 2012a). A subsequent laboratory study demonstrated the transstadial transmission of *H. canis* from larvae to nymphs of *Rhipicephalus* sp. I (Giannelli et al., 2013). These findings suggested a high transmission efficiency for this tick population of southern Italy designated as *Rhipicephalus* sp. I. On the other hand, evidence from Brazil indicates that the tropical lineage of *R. sanguineus* s.l. may not be a vector of *H. canis* (Demoner et al., 2013). Indeed, a recent study molecularly assessed the presence of *H. canis* in 81 ticks from around the world belonging to the tropical lineage of *R. sanguineus* s.l. and none of them was positive (Latrofa et al., 2014). In contrast, ticks belonging to the temperate lineage of *R. sanguineus* s.l. as well as ticks identified as *Rhipicephalus* sp. I and *Rhipicephalus* sp. III were found positive. Altogether, the aforementioned studies indicate the existence of distinct populations (or sibling species) of *R. sanguineus* s.l. around the world whose vector competence for *H. canis* may vary.

Cases of apparent tick paralysis were reported in the same animal shelter in southern Italy where the outbreak of canine hepatozoonosis was detected, and the so-called *Rhipicephalus* sp. I was the only tick found on dogs (Otranto et al., 2012a). Incidentally, cases of tick paralysis in dogs have usually been associated with other tick species (e.g., *Ixodes holocyclus*) (Webster et al., 2013) and only a single case was attributed to '*R. sanguineus*' in a dog from Venezuela (Viloria, 1954). The possible occurrence of such an outbreak of tick paralysis (Otranto et al., 2012a) would suggest a higher capacity of inducing toxicosis for this tick population present in southern Italy (i.e., *Rhipicephalus* sp. I), and raises questions as to whether genetically distinct *R. sanguineus* s.l. populations may differ in their capability to induce toxicosis in dogs.

Variations in terms of vector competence also seem to occur with other pathogens. For example, studies conducted in Uruguay and Japan investigated the occurrence of *Ehrlichia canis* infection in *R. sanguineus* s.l. ticks collected on dogs in urban and suburban areas but all ticks tested were negative (Inokuma et al., 2003; Venzal et al., 2007). Conversely, *R. sanguineus* s.l. ticks collected from dogs in several other countries have been found naturally infected with *E. canis* (Murphy et al., 1998; Ybañez et al., 2012). It is worth mentioning that the pathogen infection rate in ticks also depends on the methods used for their detection, feeding status of ticks, period of the year ticks were collected, infectious status of dogs, and number of ticks tested. A study conducted in Brazil reported *E. canis* infection rates ranging from 0 to 6.2% in *R. sanguineus* s.l. from different localities (Aguar et al., 2007). Remarkably, a recent experimental study investigated the competence of four South American populations of *R. sanguineus* s.l. in transmitting *E. canis* to dogs and provided evidence indicating that while ticks from south-eastern Brazil were competent vectors of *E. canis*, those from Argentina, Uruguay and south of Brazil were not (Moraes-Filho et al., 2012). Furthermore,

although traditionally associated to disease in dogs, *E. canis* has been implicated in cases of ehrlichiosis in humans in Venezuela (Perez et al., 2006). It remains to be clarified whether the Venezuelan population of *R. sanguineus* s.l. is more attracted to humans than other Latin American populations (Dantas-Torres et al., 2006).

Another key example is represented by *Anaplasma platys*, a rickettsial agent that infects the platelets of dogs worldwide. An experimental study failed to demonstrate that a North American strain of *R. sanguineus* s.l. is able to transmit *A. platys* (Simpson et al., 1991). However, circumstantial evidence indicates that *A. platys* may be transmitted by *R. sanguineus* s.l. (Inokuma et al., 2000; Sanogo et al., 2003). For example, a recent study suggested *Rhipicephalus* sp. I as a putative vector for *A. platys* in southern Italy (Ramos et al., 2013). Once again, these findings suggest that there might be some strain- or population-related differences in terms of vector competence of *R. sanguineus* s.l. ticks for *A. platys*.

4. Concluding remarks and perspectives

The vast geographical distribution of *R. sanguineus* s.l. ticks may be attributed to its adaptability to different climate conditions and to their close association with the domestic dog, with which they have travelled around the world. However, evidence indicates that some ticks identified as '*R. sanguineus*' around the world actually belong to different species that have long been overlooked by tick taxonomists, probably due to the lack of a *bona fide* morphological definition for *R. sanguineus* s.s.

Rhipicephalus has long been considered a problematic genus (Hoogstraal, 1956), due to the morphological similarities among closely related species as well as due to the intraspecific variations within populations of the same species. Particularly, the taxonomy of *R. sanguineus* s.s. and some closely related species is still an issue of ongoing debate. Neumann, for example, divided this species into three subspecies: *R. sanguineus sanguineus*, *R. sanguineus punctatissimus*, and *R. sanguineus brevicollis* (Neumann, 1911). These subspecies have been regarded as *incertae sedis* in Guglielmone and Nava (2014).

Recent morphological and phylogenetic studies have added weight to the hypothesis on the existence of a cryptic species-complex under the name '*R. sanguineus*'. Only a consensual definition of *R. sanguineus* s.s., based on both morphological and genetic data, will allow taxonomists to assess the validity of other species belonging to this group, through the re-examination of type specimens available in reference collection as well as through the collection and examination of new tick material collected in type localities.

Beyond taxonomy, a better definition of the so-called *R. sanguineus* group will be fundamental for the understanding of the transmission pattern and distribution of several tick-borne diseases of dogs and humans, such as Mediterranean spotted fever and Rocky Mountain spotted fever, as well as for predicting the emergence of new zoonotic pathogens (e.g., *E. canis* in Venezuela). Moreover, it remains to be addressed whether the interaction of *R. sanguineus* s.l.

with different host species has influenced the encounter and the adaptation of this tick to different pathogens.

Several attempts have been made to find better criteria for species definition in the genus *Rhipicephalus*. We endorse the words of Hoogstraal, who nearly half a century ago stated that "*problems of morphological and biological criteria for this genus are not likely to be solved until more exhaustive field research and patient laboratory investigation have been devoted to them*" (Hoogstraal, 1956).

Conflict of interest statement

The authors declare that they have no conflict of interests.

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