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2 **Soft ticks perform evaporative cooling during blood-feeding**

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31

32 **Abstract**

33 Feeding on the blood of warm-blooded vertebrates is associated to thermal stress in  
34 haematophagous arthropods. It has been demonstrated that blood-sucking insects protect their  
35 physiological integrity either by synthesising heat-shock proteins or by means of  
36 thermoregulatory mechanisms. In this work, we describe the first thermoregulatory  
37 mechanism in a tick species, *Ornithodoros rostratus*. By performing real-time infrared  
38 thermography during feeding on mice we found that this acarian eliminates big amounts of  
39 fluid (urine) through their coxal glands; this fluid quickly spreads over the cuticular surface  
40 and its evaporation cools-down the body of the tick. The spread of the fluid is possible thanks  
41 to capillary diffusion through the sculptured exoskeleton of *Ornithodoros*. We discuss our  
42 findings in the frame of the adaptive strategies to cope with the thermal stress experienced by  
43 blood-sucking arthropods at each feeding event warm-blooded hosts.

44

## 45 1. Introduction

46 Many arthropod species have adopted the blood of endothermic vertebrates as a main  
47 or sole source of food. Blood is rich in nutrients and, except for the potential presence of  
48 pathogens, otherwise sterile. Yet, obtaining a blood meal and handling it is far from being an  
49 easy task for these animals. First, the blood flows inside vessels hidden under the skin of active  
50 and relatively much bigger animals, which are capable of defending themselves or even predate  
51 the aggressor, constitutes the first risky step of blood-feeding. Second, ingesting relatively  
52 huge amounts of hot blood, represents a major challenge for blood-sucking arthropods as it can  
53 lead to heat stress and challenges physiological balance (Benoit et al. 2019; Lehane 2005;  
54 Sterkel et al. 2017).

55 Among blood-sucking Acari, two different feeding strategies are found. While  
56 Ixodidae hard ticks attach to the host skin and slowly consume the host blood for long periods  
57 that last several days, Argasidae soft ticks ingest large amounts of blood in a relatively short  
58 time (McCoy et al. 2010). In contrast to Ixodidae, in which salivary glands play a major role  
59 in eliminating water ingested during blood meal (Kim et al. 2017), argasid ticks eliminate the  
60 excess of water in their blood meal *via* their coxal gland (McCoy et al. 2010; Sonenshine 2013).

61 In the present study, we focused on the soft tick *Ornithodoros rostratus* (Aragão, 1911)  
62 as experimental model. *O. rostratus* is an argasid tick that uses an eclectic array of food  
63 sources, including dogs, pigs, cows, peccaries and humans (Cançado et al., 2008; Hoogstraal,  
64 1985; Ribeiro et al., 2013). These soft ticks are of medical and veterinary importance as they  
65 give a painful and itchy bite (Aragão, 1936; Estrada-Pena and Jongejan, 1999), due to their  
66 salivary compounds and telmophagous feeding mode, *i.e.* gathering the blood pooled in a  
67 provoked wound (Mans and Neitz, 2004). They may carry and transmit pathogens such as  
68 *Rickettsia rickettsii* and *Coxiella burnetii*, the causative agents of the Rocky Mountain spotted  
69 fever and acute Q fever, respectively, to humans and other animals (Almeida et al., 2012;

70 Hoogstraal, 1985). This species of ticks has been reported in four countries in South America:  
71 Argentina, Bolivia, Brazil and Paraguay (Barros-Battesti et al., 2006; Guglielmone et al.,  
72 2003). During its life cycle, this species has one larval and three to six nymphal instars, and  
73 adults can arise from 3<sup>rd</sup> to 6<sup>th</sup>-instar nymphs (Costa et al. 2015). This species is an obligatory  
74 hematophagous arthropod in all stages and remains in contact with its host for a period varying  
75 from a few minutes to hours (nymphal instars and adults), or even days (larvae) (Costa et al.,  
76 2015, 2016; Lavoipierre and Riek, 1955; Ribeiro et al., 2013). Although contact with their  
77 host is for a short period of time as compared to hard ticks, it remains crucial as their success  
78 in obtaining blood will then affect their survival, development and reproduction (Anderson and  
79 Magnarelli, 2008). Additionally, the shorter the contact, the lower is the risk of being perceived  
80 and killed by the host (Rossignol et al., 1985). Importantly, it is during blood feeding that  
81 pathogens circulate between vectors and their hosts, thus interfering with the epidemiology of  
82 vector-borne diseases.

83 Evidence coming from different species of haematophagous insects, including  
84 mosquitoes and bedbugs, shows that the quick intake of a large quantity of fluid much warmer  
85 than the arthropod itself is associated to thermal stress (Benoit et al. 2011). Different  
86 mechanisms are activated at each feeding event, to counteract the deleterious effects associated  
87 with the ingestion of a warm blood meal and the risk of overheating: 1) the rapid synthesis of  
88 Heat Shock Proteins (HSPs) and 2) the activation of thermoregulatory mechanisms (Benoit et  
89 al. 2011, 2019; Lahondère and Lazzari, 2012; Paim et al. 2016; Lahondère et al. 2017). Even  
90 though insects and acarions certainly differ in their anatomical and physiological  
91 characteristics, we hypothesize that they are both exposed to thermal stress during blood  
92 feeding. The question arises then, about how they avoid the potential deleterious effects  
93 associated with feeding on warm-blooded vertebrates. It has been shown that several HSPs,  
94 including HSP70 and HSP90, are greatly upregulated in the *Ornithodoros moubata* (Murray,

95 1877) midgut after feeding (Oleaga *et al.*, 2017). Moreover, in hard ticks, it is known that  
96 HSPs are overexpressed during feeding and that they play a crucial role in the mechanisms  
97 evolved by ticks in order to survive during blood feeding and to tolerate pathogen infection  
98 (Guilfoile and Packila, 2004; Oleaga *et al.*, 2017).

99 To investigate whether soft ticks have developed any thermoregulative strategy to avoid  
100 overheating during blood-feeding, we analysed the evolution of the body temperature of ticks  
101 while feeding on a host, using real-time infrared thermography. Based on these results, we  
102 then evaluated the role that the cuticle wrinkled architecture could play in thermoregulation  
103 in the soft tick *O. rostratus*.

104

## 105 **2. Material and methods**

### 106 **2.1 Ticks**

107 *Ornithodoros rostratus* (Aragão, 1911) ticks used in the experiments have been reared  
108 in the laboratory for five years from specimens collected in Nhecolândia, Mato Grosso do Sul,  
109 Brazil (19° 03' S, 56° 47' W) (Costa *et al.*, 2015). Ticks were maintained inside an incubator  
110 at 28±2°C temperature and 85±10% humidity. They were fed on Swiss mice (*Mus musculus*)  
111 every 20 days.

112

### 113 **2.2 Blood-feeding assays and thermography**

114 Fourth instar nymphs, starving from 20 to 30 days, were fed on an anaesthetised hair-  
115 less mouse (150 mg kg<sup>-1</sup> ketamine hydrochloride, Cristalia, Itapira, SP, Brazil and 10 mg kg<sup>-1</sup>  
116 xylazine, Bayer S.A., São Paulo, SP, Brazil; intraperitoneally) placed over a heating pad, to  
117 avoid drug-induced hypothermia. The temperature of each tick and the host were  
118 simultaneously recorded during the whole feeding process by means of real-time infrared  
119 thermography. A thermographic camera (Pyroview, Dias Infrared Systems, Germany)

120 equipped with a macro lens was used (Fig. 1, see Lahondère and Lazzari, 2012 for further  
121 details). This non-invasive procedure allowed recording thermographic videos, unravelling the  
122 dynamics of changes in the temperature of the tick across its body without disturbing it while  
123 feeding on the host. Videos were then analysed using the Pyrosoft software. All experiments  
124 were performed in a room maintained at  $25^{\circ}\pm 1^{\circ}\text{C}$  and 40 individual blood-feeding recordings  
125 were obtained.

126

### 127 ***2.3 Fluorescein assays***

128 In order to analyse to what extent the architecture of the tegument could influence heat  
129 dissipation, we performed an experiment in which we applied drops (approximately 10  $\mu\text{L}$ ) of  
130 a fluorescent solution composed of 1% fluorescein (Sigma-Aldrich) in saline solution on the  
131 cuticular surface of ticks ( $N = 5$ ), and monitored its spread by observing its fluorescence under  
132 UV-light excitation using an epifluorescence microscope and low magnification.

133 All procedures were done in accordance with the manuals for experiments using  
134 animals and were approved by the Ethics Committee for the Use of Animals (CEUA, Instituto  
135 de Ciências Biológicas, Universidade Federal de Minas Gerais) under the protocol 301/2013.

136

## 137 **3. Results**

### 138 ***3.1 Feeding on a live host***

139 Once placed on the anaesthetised mouse, ticks inserted their mouthparts in the host skin  
140 and remained immobile. Not all individuals could be followed from an initial biting to full  
141 engorgement. Some ticks fed only partially and left the host; others detached and bit again  
142 and, in a few cases, movements did not allow us to keep a proper focus with the thermographic  
143 camera, which lead to a decrease in reliable temperature measurements. Engorgement was  
144 noted when the tick's body became gradually rounder. After  $8.2\pm 1.7$  min (mean $\pm$ s.e.m.,  $n= 7$ ),

145 a drop of fluid appeared on the ventral part of the tick and became gradually larger, due to the  
146 continuous secretion of the coxal glands (Lavoipierre and Riek, 1955; Kaufman et al. 1981,  
147 1982). This fluid continued flowing until the end of the feeding.

148

### 149 **3.2 Blood-feeding assays and thermography**

150 When a tick was over the skin of a mouse, given its small body mass and the close  
151 contact surface (the whole ventral side), the temperature of the tick quickly increased to a value  
152 close to the temperature of its host (34°-35°C). When feeding started, the body temperature  
153 either slightly increased with blood intake or remained relatively constant (Fig. 2). When fluid  
154 excretion began, the temperature of the tick gradually decreased to reach a minimum by the  
155 end of feeding, around 3°C lower than the initial value. (Fig. 3, Supp. Video).

156 The Figure 4 depicts the difference between the temperature of the tick and that of the  
157 host, before and after the secretion of fluid began. We found that the excretion of fluid led to  
158 a significant decrease of the tick body temperature (Fig. 4).

159

### 160 **3.3 Fluorescein assays**

161 Given the particular architecture of the tegument of *Ornithodoros* along with the  
162 synchrony between secretion of fluid and temperature decrease, we suspected that a process of  
163 evaporative cooling could be involved. We also hypothesised that it would be facilitated by  
164 capillary diffusion through the three-dimensionally anfractuous structure of *Ornithodoros*  
165 cuticle (e.g. Labruna et al., 2016; Muñoz-Leal et al. 2016, 2017). Besides, the fact that  
166 temperature changes were observed to be simultaneous and similar over the whole body (*i.e.*,  
167 no heterothermy was observed, Fig. 3), suggested that fluid evaporation could occur over the  
168 whole tegument surface.

169 To test this hypothesis, we observed the dynamics of spread of a fluorescent solution  
170 in different individuals (n = 5). Figure 5 highlights two images of the same individual taken  
171 30s apart, illustrating the rapid increase of the fluorescent area by capillarity, and representative  
172 of what was observed in all tested individuals.

173 We can then conclude that the spread of the excreted fluid over the cuticular surface  
174 lead to an increase of the evaporative surface, thus facilitating the cooling of the acarian during  
175 blood-feeding.

176

#### 177 **4. Discussion**

178 The fact that haematophagous arthropods are exposed to thermal stress at each feeding  
179 event was only recently revealed, along with the existence of molecular recovery strategies and  
180 thermoregulatory mechanisms to avoid overheating during blood-feeding (Benoit *et al.* 2011,  
181 2019; Lahondère and Lazzari, 2012; Lahondère *et al.* 2017).

182 Among acarians, the synthesis of HSPs induced by heat shocks and blood-feeding in  
183 has been characterised in the hard tick *Ixodes scapularius* (Guilfoile and Packila, 2004; Busby  
184 *et al.* 2012), and more recently in the soft tick *O. moubata* (Oleaga *et al.* 2017). A functional  
185 relationship between thermal stress during feeding and HSP synthesis can thus be hypothesized  
186 to be a general mechanism in blood-sucking arthropods associated to endothermic hosts  
187 (Pereira *et al.* 2018).

188 Until now, thermoregulatory mechanisms associated with blood-feeding were only  
189 known to exist in insects (Lahondère and Lazzari, 2012, Lahondère *et al.* 2017). Our present  
190 study in *O. rostratus* not only highlighted a new ectothermic species possessing  
191 thermoregulatory abilities, suggesting that it may be also present in other acarians, but also  
192 revealed a new original mechanism, based on morphological and physiological specific  
193 adaptations. The location and intense activity of the coxal gland during feeding, as well the



194 cuticular anfractuositities of the tegument of *Ornithodoros* ticks, both contribute to the rapid  
195 dissipation of heat excess. The spread of the fluid over a large tegument surface, facilitates the  
196 heat exchange between the fluid and the body, as well as the evaporation of the fluid, reducing  
197 the body temperature of the tick by evaporative cooling. Interestingly, Yoder *et al.* (2009) have  
198 shown that dermal gland (*sensilla sagittiformia*) secretions in the brown dog tick *Rhipicephalus*  
199 *sanguineus* are implicated in heat tolerance. Indeed, the secretion and spread of this liquid on  
200 the cuticle allow this species to avoid heat stress while feeding on their host. As the exact way  
201 this gland secretion is involved in heat tolerance remains unclear, testing for the hypothesis of  
202 evaporative cooling during blood-feeding in this species could shed some light on the fine  
203 mechanisms underlying the role of these secretions in heat tolerance in the *R. sanguineus* ticks.  
204 It has also been suggested that the folded architecture of the *Ornithodoros* ticks is a derived  
205 character from their ancestor, *Nuttalliella namaqua* allowing the tick to greatly expand its  
206 abdomen and ingest an important quantity of blood quickly (Klompen and Oliver, 1993; Mans  
207 *et al.* 2011). This species is lacking coxal glands but instead possesses an anal pore, through  
208 which excretion occurs. Whether these ticks might use evaporative cooling during feeding  
209 remains unknown, but would be interesting to investigate in order to have a better  
210 understanding of the evolution of thermoregulatory processes among blood-sucking insects  
211 and acarians.

212         This is the first time that a thermoregulatory mechanism is unravelled in acarians.  
213 Among insects, evaporative cooling has been shown to occur in several species including  
214 aphids (Mittler, 1958), honeybees (Heinrich, 1979; 1980) and moths (Adams and Heath, 1964).  
215 Honeybees and moths regurgitate droplets of nectar that they maintain on their mouthparts to  
216 cool down their head thus avoiding overheating due to either high ambient temperature or  
217 activation of their flight thoracic muscles (*i.e.* endothermy) (Adams and Heath, 1964; Heinrich,  
218 1979). Moreover, honeybees move the droplets in and out of their mouth to cool down their

219 head more efficiently (Heinrich, 1980). They are thus able to decrease their head temperature  
220 by 3°C and 4°C below ambient temperature, respectively. Aphids cool down their abdomen  
221 by excreting honeydew and by keeping a typical posture (*i.e.* away from the substrate) which  
222 increases air movement around their abdomen (Mittler, 1958). Among blood-sucking insects,  
223 *Anopheles* mosquitoes are known to use droplets composed of urine and undigested blood to  
224 cool down their abdomen during feeding (Lahondère and Lazzari, 2012). Soft ticks ingest  
225 blood up to 12-fold their own body weight and to restore their water balance as well as to  
226 concentrate the nutrients in the ingested blood, they excrete large amount of coxal fluid which  
227 is hypoosmotic. This fluid, unlike anopheline mosquitoes which might also excrete fresh  
228 erythrocytes (Briegel and Rezzonico, 1985), is mainly composed of water, electrolytes, nucleic  
229 and amino acids, proteins, carbohydrates and lipids (Frayha *et al.*, 1974). It is worth  
230 mentioning that coxal fluid is excreted by soft ticks when exposed to temperatures over 40°C  
231 (Kaufflan and Sauer, 1982; Rémy, 1922) thus reinforcing the hypothesis that coxal fluid  
232 excretion participates in thermal stress management in these ticks.

233 Finally, evaporative cooling is not the sole mechanism for thermoregulation that has  
234 been described in haematophagous insects. Indeed, the kissing bug, *Rhodnius prolixus*,  
235 possesses in its head an elaborated counter-current heat-exchanger, which reduces the  
236 temperature of the ingested blood, before it reaches the abdomen of the bug (Lahondère *et al.*,  
237 2017). It is then possible, that other tick species could have developed other strategies for  
238 reducing thermal stress during blood feeding.

239

## 240 **5. Conclusions**

241 Our study shows for the first time in acarians, that soft bodied ticks perform  
242 thermoregulation during feeding by excreting fluids through their coxal gland that spreads to  
243 their cuticle and cool them down. These results extend previous knowledge obtained in insects,

244 to other blood-sucking arthropods. We have just began disentangling how blood-sucking  
245 arthropods manage avoiding the deleterious effects associated with feeding on endothermic  
246 vertebrates, and much work remains to be conducted to get a full picture of the spectrum of  
247 possible adaptations developed by these arthropods during their long evolutionary history.

248

249

#### 250 **Author contribution**

251 Experimental design: C.R.L, C.L. and M.H.P.

252 Thermographic recordings: C.R.L.

253 Data Analysis: A.F. and C.R.L.

254 Tick rearing and expertise: R.N.A.

255 Fluorescent microscopy: M.H.P.

256 Manuscript draft: C.R.L.

257 Final elaboration and editing of manuscript: all the authors

258

#### 259 **Conflict of interest**

260 There is no conflict of interest.

261

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270

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395 **Figure captions**

396

397 **Figure 1.** Experimental setup for real-time thermographic recording of the dynamics of  
398 temperature changes in an *Ornithodoros rostratus* nymph feeding on an anaesthetised  
399 hairless mouse. A thermographic camera equipped with a macro lens, was connected to a  
400 laptop computer to record the evolution of the tick body surface temperature.

401

402 **Figure 2.** Sample record of the temperature of a tick during feeding. **A.** Temperature on the  
403 tick body surface. **B.** Temperature difference between the tick and the mouse. Red arrows  
404 indicate the moment when the secretion of coxal fluid began.

405

406 **Figure 3.** Thermographic images of a tick, before fluid secretion (top) and during fluid  
407 secretion (bottom). Different colours indicate different temperatures, as indicated in the  
408 reference scale on the right. A drop of coxal fluid (in green) can be observed in the ventral  
409 region of the tick in the bottom image.

410

411 **Figure 4.** Temperature difference between the tick and the host before and after the secretion  
412 of coxal fluid began. The statistical comparison rendered a highly significant difference  
413 (paired Student *t*-test,  $p < 0.01$ ,  $n = 7$ ).

414

415 **Figure 5.** **A.** Dorsal and **B.** Ventral views of an adult *Ornithodoros rostratus*. Circles indicate  
416 the opening of coxal glands. **C-D.** Sample record of the spread of a drop of fluorescein  
417 solution over the dorsal cuticular surface of a tick, immediately after dropping (left) and  
418 30s later (right).

419

420 **Supplementary material**

421

422 **Movie 1.** Example of a thermogram of an *Ornithodoros rostratus* nymph during blood-feeding  
423 on a hairless mouse. At the beginning of the process, the temperature of the tick increases  
424 as a consequence of blood ingestion. Then, the accumulation of fluid can be observed in  
425 the anterior (right) part of the body, followed by a decrease in the temperature of the acari.  
426 The sequence has been accelerated, in order to present a process usually taking around 30  
427 min.











