The Porto Santo Island Hyalomma sp. A study based on its morphology and metries of

the dorsal scutum. 1 The males

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Resumo

Foram morfológica e biometricamente comparadas uma amostra de machos Hyalomma colhidos na ilha do Porto Santo e uma amostra de machos identificados como Hyalomma lusitanicum presentes na carracoteca do Departamento de Sanidade Animal e Vegetal da Universidade de Évora. Na comparação biométrica recorreu-se às dimensões máximas, comprimento e largura, do escudo dorsal. Observou-se em ambas as amostras uma ampla e similar variabilidade morfológica, tanto no que concerne às dimensões máximas do escudo dorsal, como no que diz respeito às várias características morfológicas de natureza qualitativa, com exemplares de grande e de muito pequenas dimensões. Não foram observadas diferenças qualitativas entre as amostras estudadas. Não foram observadas diferenças significativas entre as dimensões máximas do escudo dorsal das amostras. O comprimento e a largura máxima distribuem-se normalmente em ambas as amostras estudadas. Verificou-se que as dimensões comprimento e largura máxima do escudo dorsal são linearmente dependentes. Foram encontradas diferenças significativas entre os respectivos coeficientes angulares. Conclui-se que na ilha do Porto Santo só está presente uma espécie de Hyalomma sp, Hyalomma lusitanicum. Esta espécie mostra uma grande variabilidade morfológica, sendo os machos das populações da ilha do Porto Santo e os presentes na carraçoteca da Universidade de Évora qualitativamente indistinguíveis. Estes são metricamente diferenciáveis ao nível do comprimento e largura do escudo dorsal. Quando provenientes da ilha do Porto Santo o escudo dorsal é comparativamente mais estreito. Levanta-se a hipótese de tratar-se de uma adaptação local das carraças principalmente provenientes do espaço de Portugal continental. Não se exclui a hipótese das diferenças advirem da introdução de genes de espécies próximas provenientes do acasalamento com sucesso com formas introduzidas pela avifauna proveniente do norte de África.

Abstract

Had been morphologically and bio-metrically compared male *Hyalomma* ticks samples from Porto Santo island and from the University of Évora coillection. The sec ond sample had been identified as *Hyalomma lusitanicum*. In the metric comparison have been studied the maxim length and width of the male dorsal scutum. Had been not found any differences at qualitative level. Both samples shown a high degree of variability, both for the morphological and metrical levels, with big and very small

specimens. Had been not found any significant differences at metric level in what concerns to the maxim length and width of the male dorsal scutum. In both samples length and width are linearly related. We concludes that in Porto Santo island there is only one *Hyalomma* sp specie, *Hyalomma lusitanicum*. We also concludes that *Hyalomma lusitanicum* it's a specie with a high degree of morphological variability. Nevertheless had been found significant differences at the angular coefficient level, with the Porto Santo specimens dorsal scutum comparatively narrowest. These differences may came from a local adaptation of the *Hyalomma lusitanicum* ticks mainly proceeding from Portugal main land, or alternatively, from the introduction of foreign genes proceeding from closed species ticks brought from North of Africa by bird path way.

Introduction

Hyalomma Koch, 1844 is known in Portugal by the presence of the species *Hyalomma marginatum marginatum* Koch, 1844, and *Hyalomma lusitanicum* Koch, 1844, respectively in Portugal main land (Dias, 1994) and Portugal main land (Dias, 1994) and Madeira Island (Almeida, 1998). In addition to these two species, for the Iberian peninsula, are also identified *Hyalomma anatolicum excavatum* Koch 1844, *Hyalomma detritum detritum* Schulze, 1919 and *Hyalomma impressum impressum* Koch 1844 (Dias, 1994). Nevertheless the presence of the last specie is questionable (Dias, 1994) attending to the acknowledged geographic distribution, a West African tick not described to the Mediterranean littoral (Hoogstraal, 1956). Also the presence of many of the other species it is refutable, being, for some authors, nothing more than incorrect identifications. In fact the Iberian *Hyalomma* sp would be constituted practically only by *H lusitanicum* and *H. marginatum marginatum*.

Being a hot, dry and poorly afforested territory, Porto Santo island has good climatic conditions to *Hyalomma* sp proliferation. Besides, the presence of *Bos taurus* and *Ovies aries*, associated whit great populations of *Oryctolagus cuniculus*, a well known immature, but apparently not imago (Ouhelli & Pandey, 1984), instar host, *Rattus* sp. and several species of birds, it's advantageous to the genera. In fact the presence of *Hyalomma* sp in Porto Santo Island was for us known since sometime ago, had been collected in *B. taurus*, *O. aries* and even in *Capra hircus*. Interestingly we never found the genera in *Canis familiaris*, known as host both for *H. lusitanicum* and

H. marginatum (Dias, 1994). Notwithstanding we had never attained a specific identification.

Being the Porto Santo island bio-geographically influenced by the Iberian peninsula, specially Portugal, through cattle movements, and by the Mediterranean and Northwest of Africa by it's geographic location, namely via migratory and transported by storms birds path way, it was possible the jointly presence of Mediterranean, African and Iberian species. That is to say *H. lusitanicum* and *H. excavatum*, the small *Hyalomma* (Hoogstraal, 1956), according to Feldman-Muhsam (1954) synonym of *H. anatolicum excavatum*, pretty frequent in Paleartic Africa, namely in Libya (Hoogstraal & Kaiser, 1960) and Morocco (Bailly-Choumara et al., 1974). As a matter of fact this specie it is well enough distributed in Mediterranean basin, having records from South of Russia and from Palestine to Mauritania and Spanish Sahara (Dias, 1994). Interestingly, *H. anatolicum excavatum* are not properly small sized *Hyalomma*. In fact Feldman-Mubsam (1954) states that the male dorsal scutum maxim dimensions for the Koch type it's of 4,0 mm of length and 2,6 mm of width, somehow bigger in width than the stated by Hoogstraal (1956).

The observation of the material collected pointed out only to the presence of *H*. lusitanicum. Nevertheless the ample morphological variability observed, along with a deep morphometric variableness, with both very small and fair large individuals, suggested the need of a more deep analysis, as long as since the Delpy (1936) and Adler & Feldman-Muhsam (1946, 1948), quoted by Feldman-Muhsam (1954), works it might be perfectly established the wide range of morphological variation inside the Hyalomma sp good species, namely at the colour of the legs, the form of parma and stigma, and minute variations in the punctation of the scutum. Moreover, the absence of visible lateral grooves, even very short, according with Dias (1994) discriminant between H. lusitanicum and H. excavatum, somehow puts away the possibility of being involved H. excavatum specimens. Interestingly Feldman-Muhsam (1954) and Hoogstraal (1956), had synonymised H. lusitanicum Koch, 1844, with H. excavatum Koch, 1844, synonym of *H. anatolicum excavatum*. In order to establish the correct systematic position of the material, we considered necessary to perform a morphologic and metric comparison of the specimens collected in the Porto Santo island and elsewhere. In this work we presents the morphologic description and the comparison of the Porto Santo male Hyalomma sp. and the Hyalomma lusitanicum male from Portugal main land, preserved at the Évora university.

Material and methods

A sample of 164 *Hyalomma* sp. male specimens, randomly selected from the material collected in soil samples, *Bos taurus*, *Ovies aries* and *Capra hircus* from January to July 2001 in Porto Santo island, and briefly identified as *Hyalomma lusitanicum*, was morphologically and metrically compared with a sample of 171 *Hyalomma lusitanicum*, removed from soil samples and *Bos taurus* in Baixo Alentejo, Portugal, randomly chosen from the material preserved in the Évora University. The microscope metric comparison layed upon the maximum length, from scapulae to the central festoon, and width of the dorsal scutum. The used number of individuals was drawn from a smaller sample mean in order that the mean parametric variation from the statistic mean were equal or less than 3% at 99% of confidence, assuming a normal data distribution. All the specimens used were being preserved in 70% alcohol with or without glycerine. The statistical analysis where performed using the "Statistica" program.

Results

1. Description of the Porto Santo male Hyalomma

Long rostrum with rectangular basis capitulum without cornua evident. Dark brownish honey colour scutum without the post preservation evident presence of any enamel cover areas near by the scapulae or the median field. Usually rare large and me dium size punctations mainly focused near by the scapulae and anterior median field, between the cervical grooves. Some times the specimens are densely punctuated. Usually strong and evident depression of the posterior part of the scutum between the elevations formed by the fusion of the festoons one to three. In some individuals are less evident both the fusion of the festoons and the depression of the scutum. This depression is densely punctate with small sized punctations, being possible to find some few and scattered large punctations. Absence of evident lateral grooves, in fact always obsolete in all specimens but one, where went possible to find a very superficial and hardly detectable lateral grooves, almost reaching the leg IV level. In some individual it's possible to find a line of punctations that continues anteriorly from the lateral grooves. In neither case was found a line of punctations groove-like enough to confuse with lateral grooves. Posteromedian groove deep at rear, reaching or not the central festoon limiting grooves, and anteriorly superficial, almost gaining the fovea proximity.

Absence of parma, intended as the non chitinous central festoon. Absence of bridge over the central festoon. The central festoon usually as the same dark brownish honey colour scutum, although some specimens showed a partially pale colour due to an in complete chitinous coating. Paramedian grooves obsolete, although in one quite flattened specimen they were pretty evident. Tegument of the same colour of the scutum or pale, light brownish honey colour. Coxa I typically sub-genus Hyalomma, deeply divided into a narrow external branch and a wider internal branch. Legs, usually, but not always, specially in smaller specimens, each time stronger from leg I to leg IV, less dark brownish honey than the scutum, with a spotted pale honey enamel pattern not particularly evident post conservation in 70% alcohol Adanal shields extending them selves in a narrow and ticklish tip behind the anus. Tegument from dark brownish honey colour, undistinguishable from scutum, to pale brownish honey colour. Accessory shields present. Presence of subadanal shields in line with the central axis of the adanal shields, even in the engorged individuals. Scutum restraining at the spiracular plates level pretty evident in some specimens but not in others. Spiracular plates of normal male type, with long "tail". Not hirsute circumspiracular area. From small to large sized specimens.

2. Description of the *H. lusitanicum* specimens from the material preserved in the Évora University

The description of the material preserved in the Évora University it's similar to the above description of *Hyalomma* from Porto Santo, with the exception of the lateral grooves were always not evident, obsolete.

3. Metrics of the dorsal scutum

Porto Santo Male *Hyalomma lusitanicum* scutum length: N = 164. Average = 3,486 mm. Variance 0,113 mm. Maxim = 4,191 mm. Minim 2,540 mm. Differences not significant to the data adjustment to the normal distribution. Significant adjustment.

 X^2 = 3,462017; gl = 2; p = 0,1771223.

Évora University male *Hyalomma lusitanicum* collection scutum length: N = 171. Average = 3,588 mm. Variance 0,101 mm. Maxim = 4, 3 18 mm. Minim 2,667

mm. Differences not significant to the data adjustment to the normal distribution. Significant adjustment. $X^2 = 2$, 7496 13; gl = 2; p = 0,2529035. Absence of significant differences (p<0,01) between the two analysed samples. t = -2, 84163; gl = 333; p = 0,004765; N₁ = 164; N₂ = 171.

Porto Santo Male *Hyalomma lusitanicum* scutum width: N = 164. Average = 2,206 mm. Variance 0,061 mm. Maxim = 2,794 mm. Minim = 1,524 mm. Differences not significant to the data adjustment to the normal distribution. Significant adjustment. $X^2 = 3,194638$; gl = 1; p = 0,073887.

Évora University male *Hyalomma lusitanicum* collection scutum width: N = 171. Average = 2,348 mm. Variance 0,05 mm. Maxim = 2,794 mm. Minim = 1,778 mm. Differences not significant to the data adjustment to the normal distribution. Significant adjustment. $X^2 = 0$, 45057 17; gl = 1; p = 0, 5020685. Absence of significant differences (p<0,0l) between the two analysed samples. t = -5,55334; gl = 333; p = 0,193134; N₁ = 164; N₂ 171.

4. Correlation between maxim width and maxim length

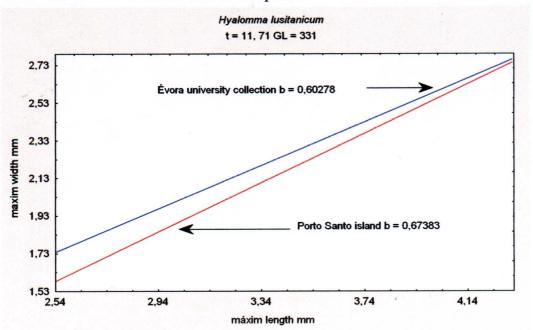
The maxim width and the maxim length are linearly dependent.

Porto Santo male *H. lusitanicum*. Maxim length = -0, 1437 + 0, 67383 x Maxim width. r = 0,91620. r² = 0,839418. t = 29,10034. N = 164. p = 0, 00 (significant at level 0,01).

Évora University collection male *H. lusitanicum*. Maxim length = -0,18567 + 0,60278 x Maxim width. r = 0,85839. r² = 0,736826. t = 21,75227. N = 171. p = 0,00 (significant at level 0,01).

Correlation straight lines with significantly different angular coefficients. t = 11, 71. gl = 331. P < 0, 01. Male *Hyalomma lusitanicum* from the Évora University collection more narrowest than Porto Santo island Hyalomma. Here graph I

Graph I



Discussion

In the generality both Porto Santo island and Évora *Hyalomma* sp. material are according with the *Hyalomma lusitanicum* description (Tendeiro, 1955), as long as being evident a pretty morphological variability.

The presence of a individual with very superficial and hardly visible short lateral grooves, almost reaching the leg IV level, could be interpreted a the presence of an *H. excavatum* specimen. In fact *H. excavatum*, the small *Hyalomma* in the Hoogstraal (1956) sense, it's very similar to *H. lusitanicum*, with the main difference of the presence of short lateral grooves restricted to the posterior third of the scutum in the first case, contrarily to obsolete ones in the second case (Dias, 1994). Nevertheless the occurrence of only one specimen with this characteristics in all the examined material take us to admit one more and rare specie variation in such a changeable genera. Addition ally the specimen size do not encourage us to assume the presence of *H. excavatum*, because, with our data, *H. excavatum*, Hoogstraal (1956) sense, and *H. lusitanicum* male scutum maxim dimensions partially overlaps. The male scutum maxim length in *Hyalomma excavatum* it's not bigger than 4,18 mm and rarely superior to 3,75 mm and the maxim male scutum width it's not superior to 2,19 mm (Hoogstraal, 1956). And the Koch type analysed by Feldman-Muhsam (1954) points to a male dorsal scutum width of bigger size, 2,6 mm, fairly near our data, maxim of 2,8 mm.

Interestingly both the maxim scutum width and length of Porto Santo island and Évora male sample specimens are different from what it's stated by Tendeiro (1955), length of 4,05 to 3,25 mm and width of 2,80 to 2,40 mm, with bigger, although with smaller maxim dimensions specimens, and less variable individuals. It's difficult to interpret these divergences in asmuch as Tendeiro (1955) do not propose the presence of any other Hyalomma specie in Portugal main land than the Hyalomma marginatum marginatum, a larger and very different form. Anyway in what concerns to the male maxim scutum dimensions there is no reasons to suppose the presence of other specie than H. lusitanicum. The other differences found, namely in what concerns to the depression of the posterior part of the scutum between the elevations formed by the fusion of the festoons one to three, that in some individuals are less evident, both at the fusion of the festoons and the depression of the scutum level, are not discussed in the consulted literature. However Hoogstraal (1956) consider that in any field collection some atypical specimens could be found. In what concerns to *H. excavatum* the author points out that it's even possible to find, in some engorged individuals, a relocations of the sub-adanal shields axis implantation, that may be displaced to a position similar to what can be seen in *H. dromedarii*.

As pointed above, the morphological variableness found in our specimens, both from Porto Santo and Évora samples, it's consistent with Hyalomma sp variability, and do not points out to the presence of more than one Hyalomma specie. The absence of significant differences with the normal distribution of the scutum metrics points out to a conspecific situation, being the variability continuous and associated with the dependence of poli-genes with addictive action. Really it's well known that different species and different subspecies, or even just allopathic populations, usually show morphologic differences at metrical level, evidenced by more than one modal point in the data frequency distribution. It elapses from the fact of the absence of mating among male and female from allopathic populations dictate different local adaptations. So, although very variable in its morphology, there is no reasons to assume the presence in Porto Santo Island, and also in the Évora preserved specimens, of male individuals from other species than Hyalomma lusitanicum Koch 1844. The significant agreement of the scutum width and length data, both from Porto Santo and Évora specimens, with the normal distribution, suggests also the absence of different adaptability of the individuals of determined size. Neither the bigger or the smaller specimens are more adaptable.

The absence of significant metrical differences between the Porto Santo island and the Évora male scutum are not unexpected since it's assumed that Porto Santo Hyalomma sp has mainly a Iberian origin, specially via Portuguese cattle movements, that are relatively recent. The island began to be colonised approximately in the early XV century. Nevertheless there is significant differences between maxim width and maxim length correlation lines at angular coefficient level, what may suggest some differential adaptation, if really existing a quick one, or even the matting, and progeny survival and subsequent reproduction, with individuals of genetically dose Hyalomma species, arrived to the island eventually by bird pathway. Although, as far as we know, not to Hyalomma sp, it's described the viability of inter-specific tick reproduction (Cooley, 1938; Thompson et al, 1981; Pegram et al, 1987; Estrada-Peña & Sanches-Acedo, 1988), even in small frequencies, with some times unfertile males and full fertile females. The indexing of the noted divergences to the differences in male repletion are not acceptable since in both samples there are a fairly similar number of engorged males. Whatever the subjacent causes are, from our data becomes established that we have in Portugal at least two allopathic populations qualitatively indistinguishable but metrically different. A Porto Santo island population and a Portugal main land population, with the smaller Porto Santo island specimens comparatively more narrow than the Évora ones.

After the morphological and metrical male *Hyalomma* analysis and in view of the above exposed, we assume that the noted broad morphological variability it's irrelevant. So, in Porto Santo Island there is only one *Hyalomma* specie, *Hyalomma lusitanicum* Koch 1844. Then, in order to identify any Porto Santo island male ticks as *H. lusitanicum*, it's enough to observe the presence of a long rostrum, anal groove contouring the anus behind, presence of eyes, coxa I bifid, deeply divided into a narrow external branch and a wider internal branch, presence of adanal, subadanal and accessory shields, the first shields in line with the second, absence of evident lateral grooves, and brownish with a spotted pale enamel patterned legs. We may also search for adanal shields extending themselves in a narrow and ticklish tip behind the anus and spiracular plates with long "tail".

Reverences

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