MORPHOLOGICAL DIFFERENTIATION OF RHIPICEPHALUS BOOPHYLUS ANNULATUS, RHIPICEPHALUS BOOPHYLUS MICROPLUS AND HYBRIDS POPULATIONS COLLECTED IN NORTHERN CÔTE D'IVOIRE

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ABSTRACT

In order to differentiate 3 different tick populations, 301 specimens of tick belonging to Rhipicephalus Boophilus genera were taken from cattle farms located in northern Côte d'Ivoire. For this work, 101 specimens of Rhipicephalus Boophilus microplus, 100 ticks of R B annulatus and 100 ticks with the characteristics of species named were submitted to a morphometric study. This study, based on 5 morphological descriptors made it possible to verify the homogeneity within and between all studied tick groups. This work confirms the natural hybridization of R B annulatus and R B microplus ticks on cattle farms in northern Côte d'Ivoire. In addition, analysis of the morphometric traits retained revealed intrapopulation heterogeneity (CV> 15%) for all 3 tick groups. On the other hand, at the multivariate analysis level, it emerged that 4 of the 5 descriptors used were significantly discriminating. It was about the waist Length (Lt), Length of the capitulum (Lca), Length of the opistosome (Lop) and Length of the legs (Lpa). These descriptors allowed highlighting the existence of 2 groups morphologically distinct from each other. These groups were constituted in one hand by R B microplus specimens and the other hand by R B annulatus ticks. The Mahanobis distance between those groups were 15.26% while those of hybrid group and each of R B annulatus and R B microplus were respectively 4.23 and 6.01. The third ticks group was morphologically closer to R B annulatus than R B microplus. Moreover, at aggregation distance 10, each group consisted of subgroups, which confirmed ticks intragroup heterogeneity but also raised questions about the possibility of hybridized ticks of different generations in each groups. This sentence must be deleted. To do this, subsequent molecular studies on specimens from these 3 tick groups would be needed to confirm this hypothesis.

KEYWORDS: Differentiation, morphometric, Rhipicephalus Boophilus, microplus, annulatus, hybrids, Côte d’Ivoire.

INTRODUCTION

Ticks are a group of ectoparasites with a global distribution containing 907 species of which 223 have been identified in Africa (Socolovschi et al., 2008). Among this parasites group, there are soft ticks and hard ticks (Ixodina) (Perez-Eid et Gilot, 1998). These hematophagous organisms, vectors of diseases, are liable to vertebrates and particularly to mammals. In ruminants, ticks are vectors of bacterial or rickettsial infectious diseases such as babesiosis, anaplasmosis, theileriosis, cowdriosis and dermatophilosis (Bowman et al., 2008). In addition to these pathologies, they can transmit to animals often deadly viral diseases such as Omsk haemorrhagic fever (Casals et al., 1969) and Crimean-Congo Fever (Mathiot et al., 1988).

In Côte d'Ivoire, the first inventory of tick species was carried out in the 1960s by Aeschlimann (1967). This author had conducted a large entomological survey on ticks infesting vertebrates across the country. As a result
of this work, further research on ticks and the diseases they transmit has been conducted in different parts of the country. Thus, in the 1990s, only 8 cattle ticks species were inventoried in the north of the country (Yapi, 1990) versus 7 taxa in 2009 by Tuo (2013). Recent works realised by Kouassi (2012) and Touré et al. (2014) throughout the northern region of the country has revealed respectively 6 and 4 ticks’ species. These works have permitted to situate a R B microplus possible introduction in this area between 2009 and 2011. Since that period a spatial distribution modification of ticks has been observed (Gragnon et al., 2018). The colonization of this taxon of the different biotopes of northern Côte d’Ivoire is often followed by strong complaints from farmers, as reported in Burkina Faso by Sanou (2012). The latter constantly evoke the strong infestation of their animals by ticks resistant to commonly used acaricides.

Today R. (Boophilus) microplus is one of the biggest threats to cattle farming in Côte d’Ivoire. The harmful impact of this species of ticks on the breeding is all the greater as it has a capacity to transmit a large number of diseases, a great capacity to develop resistance to acaricides (Andrew et al., 2003; Ducornez et al., 2005; Guerrero et al., 2012; Yessinou et al., 2018) and a very invasive character (Madder et al., 2011; De Clercq et al., 2012).

Previous works has demonstrated the ability of R B microplus to mate with other Boophilus tick species (Graham et al., 1972; Spickett et Malan, 1978). With Boophilus decoloratus, R B microplus gives sterile hybrids (Spickett et Malan, 1978). On the other hand with the R B annulatus species, the female hybrids obtained retain their ability to reproduce and produce offspring, especially when it comes to backcrossing with one of the parental species (Thompson et al., 1978). More recently in central and northern Côte d’Ivoire, it was discovered during an entomological survey of 3 couples of male R B microplus and female R B annulatus ticks (Gragnon, 2012). This observation prompted a new study to verify the existence different types of couples R B microplus X R B annulatus in that breeding zone and to characterize the different types of ticks R B encountered in this area. This study on the morphological differentiation of these 3 populations of R B falls within this framework.

**MATERIAL AND METHODS**

**Presentation of the study area**

This study was conducted in northern Côte d’Ivoire which concentrates most of the national cattle herd. It is an area under the influence of the South Sudanese climate. This zone is between 9 ° and 10.5 ° North latitude and 4 ° and 6 ° West longitude. Its climate is very hot and dry with daily temperature ranges up to 20 °C and humidity ranging from 40% to 70%. This area has a dry season from November to June, punctuated by a few rains in April and a rainy season from July to October. The average annual rainfall is 1200 mm and its vegetation consists of open woods and savannas.

**Biological material**

The study included adult female ticks not engorged belonging to Rhipicephalus Boophilus microplus, Rhipicephalus Boophilus annulatus species and females ticks possessing morphological characteristics specific to the first 2 taxa mentioned. All these ticks were collected on cattle from 5 farms in northern Côte d’Ivoire.

**Collection, identification and measurement of ticks**

For this study, 23 cattle farms identified in a previous entomological survey as infested with R B microplus and R B annulatus were re-surveyed. In these farms, 20 animals were chosen at random, without distinction of sex, age or race. On these animals the ticks were harvested in the ticks were harvested taking care to not break their rostrums. The ticks thus collected in each farm were kept in bottles containing ethanol at 70 °. Each flask has been assigned a code taking into account the date and the farm where the sampling was done. Packed ticks were transported to Korhogo LANADA where they were individually identified and counted.

In the laboratory, ticks were observed using a binocular microscope and identified on the basis of their morphological characteristics, using the identification key of Walker et al. (2003). At the end of this operation, 5 farms infested by R B microplus and R B annulatus couples were selected for this study. Of all the ticks collected in these farms, 301 adult females (101 specimens of Rhipicephalus Boophilus microplus, 100 ticks of Rhipicephalus Boophilus annulatus and 100 ticks hybrids (R B microplus X R B annulatus) were chosen for the morphometric study. The 301 ticks chosen were measured according to the method described by Boyard (2007).

Thus, for this study, these were the following linear characters; Length of the waist (L1), Length of the capitulum (Lca), Length of the podosome (Lpo), Length of the opistosome (Lop) and Length of the legs (Lpa).

**Data analysis**

Data processing was performed using elementary statistics, one-way analysis of variance (ANOVA) and multivariate analysis. Multivariate statistics include Principal Component Analysis (PCA), Discriminant Factor Analysis (DFA) and Hierarchical Classification Analysis (HCA) based on Mahalanobis distance. All of these statistical tests were performed with the STATISTICA version 7.1 software.

**RESULTS ET DISCUSSION**

**Results**

**Observations during ticks collection**

Two findings were made during this study. The first concerns the presence of male R B microplus and female
R B annulatus pairs. In addition, the hybrid specimens collected were all female.

Intragroup morphometric variability
The coefficients of variation values of morphometric variables studied ranged from 16.10% to 33.00% for all variables taken on the three populations (Table 1). All tick populations were heterogeneous for all the studied characters (CV>15%). Lt, Lpod and Lop were highest for R B annulatus population and lowest for R B microplus one. In contrast Lpa value was most important for R B microplus specimens and lowest for R B annulatus population. For Lca measurements, highest value was found in hybrids specimens while the smallest size was observed in R B microplus group.

Table 1:

<table>
<thead>
<tr>
<th>Title</th>
<th>R B annulatus</th>
<th>hybrids</th>
<th>R B microplus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± sd</td>
<td>cv</td>
<td>Mean ± sd</td>
</tr>
<tr>
<td>Lt</td>
<td>5.468±1.330</td>
<td>24.32%</td>
<td>3.990±0.643</td>
</tr>
<tr>
<td>L, CA</td>
<td>1.014±0.239</td>
<td>23.58%</td>
<td>1.171±0.223</td>
</tr>
<tr>
<td>L, POD</td>
<td>2.276±0.710</td>
<td>31.18%</td>
<td>1.236±0.270</td>
</tr>
<tr>
<td>L, OP</td>
<td>2.184±0.703</td>
<td>32.20%</td>
<td>1.576±0.432</td>
</tr>
<tr>
<td>L, PA</td>
<td>1.672±0.347</td>
<td>20.74%</td>
<td>1.558±0.270</td>
</tr>
</tbody>
</table>

The ANOVA showed that the all morphometric characters measured had very highly significantly difference (P<0.001) between the three species.

Differentiation of populations by the Principal Component Analysis (PCA)
The principal component analysis of the collected data was carried out by retaining only the axes expressing an eigenvalue equal to or greater than 0.66. For that the two first components representing 82.48% of the cumulative variances were taken into account. The correlations of the different variables with these axes are presented in Table 2. Only the first axis contributes significantly to the discrimination of the three genetic types of ticks. Indeed, all the descriptors (Lt, Lca, Lpod, Lop) are strongly negatively correlated to this factorial axis with the exception of Lpa. On the other hand, this morphometric character (r = -0.94) is negatively correlated with the factorial axis 2.

Table 2: Eigenvalues with variance percentage of the first two axes in the PCA.

<table>
<thead>
<tr>
<th>Eigenvalues</th>
<th>Axis 1</th>
<th>Axis 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Total variance</td>
<td>62.773</td>
<td>19.707</td>
</tr>
<tr>
<td>Cumul %</td>
<td>62.773</td>
<td>82.480</td>
</tr>
<tr>
<td>Lt</td>
<td>-0.989</td>
<td>-0.098</td>
</tr>
<tr>
<td>Lca</td>
<td>-0.663</td>
<td>0.211</td>
</tr>
<tr>
<td>Lpo</td>
<td>-0.885</td>
<td>-0.193</td>
</tr>
<tr>
<td>Lop</td>
<td>-0.936</td>
<td>-0.108</td>
</tr>
<tr>
<td>Lpa</td>
<td>0.244</td>
<td>-0.939</td>
</tr>
</tbody>
</table>

The Projection of all the three ticks’ populations on the principal components (PC1) and (PC2) gave Figure 2. On the scatter plot, the tick population studied is divided into two groups.
Figure 2: Projection of tick populations in the factorial axis 1 and 2 of morphometric parameters' PCA.

The specimens belonging to *R B microplus* group are entirely located in the positive part of PC1 and are characterized by high values of characters positively correlated to this axis. Those of *R B annulatus* ticks group are mostly distributed in the negative coordinates part of the factorial axis 1. These individuals display the greatest values of the descriptors correlated to factorial axis 2. The scattered plot formed by *R B annulatus* almost covers entirely that of hybrid ticks group and a small part of that of *R B microplus*. No parameter really contributes to discriminate the three groups of ticks. The factorial axis 2 contributes strongly to the characterization of the populations studied.

Differentiation of populations by the Discriminant Factorial Analysis (DFA)

Lambda Wilk test has identified on all 5 morphometric characters studied, 4 discriminating descriptors for ticks species studied (Table 3). According to the decreasing importance of their discriminating powers [wilk's lambda ($\lambda$)], there is the Lop ($\lambda = 0.2240$), the Lt ($\lambda = 0.2207$), the Lca ($\lambda = 0.2199$) and the Lpa ($\lambda = 0.2179$).

The discriminant analysis confirmed 86.38% of the classifications from the PCA (Table 4). As a result, a reclassification of some specimens from different samples analyzed has been proposed.

Table 3: p values of the different variables after the Lambda Wilk test.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Wilk (Lambda)</th>
<th>P values</th>
<th>Toler.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lt</td>
<td>0.2207</td>
<td><strong>0.0049</strong></td>
<td>0.0709</td>
</tr>
<tr>
<td>Lca</td>
<td>0.2199</td>
<td><strong>0.0081</strong></td>
<td>0.4141</td>
</tr>
<tr>
<td>Lpo</td>
<td>0.2141</td>
<td>0.4200</td>
<td>0.1750</td>
</tr>
<tr>
<td>Lop</td>
<td>0.2240</td>
<td><strong>0.0005</strong></td>
<td>0.2185</td>
</tr>
<tr>
<td>Lpa</td>
<td>0.2179</td>
<td>0.0307</td>
<td>0.9909</td>
</tr>
</tbody>
</table>

Table 4: Percentage of individuals reclassified in each group, in the validation of the DFA for the morphometric data.

<table>
<thead>
<tr>
<th>Groups</th>
<th>% Correct</th>
<th>R B microplus</th>
<th>R B annulatus</th>
<th>hybrid</th>
</tr>
</thead>
<tbody>
<tr>
<td>R B microplus</td>
<td>89.78</td>
<td>123</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>R B annulatus</td>
<td>86.04</td>
<td>0</td>
<td>74</td>
<td>12</td>
</tr>
<tr>
<td>Hybrid</td>
<td>80.77</td>
<td>4</td>
<td>11</td>
<td>63</td>
</tr>
<tr>
<td>Total</td>
<td>86.38</td>
<td>127</td>
<td>85</td>
<td>89</td>
</tr>
</tbody>
</table>

Thus, 4 hybrids tick specimens were assigned to the *R B microplus* group and 11 to the *R B annulatus* group. On the other hand no specimens of *R B microplus* were reclassified in the group of *R B annulatus* and vice versa.

Also, the reclassifications made for certain individuals of *R B microplus* and *R B annulatus* were made in the group of hybrid ticks. The results of DFA identified the same groups as obtained by the PCA (Figure 3).
According to the ticks representation based on λ-Wilk test results, *R. B. annulatus* and *R. B. microplus* populations are clearly distinct from one to another. On the other hand, a slight overlap is observed between the polygons representing hybrids population and the two others. *R. B. annulatus* specimens are mainly located in canonical axis 1 positives coordinates zone while *R. B. microplus* individuals are located in the negative coordinates of the canonical axis 2. On the other hand, the hybrid specimens are mainly oriented in the plane formed by the positive coordinates of the canonical axis 1 and the negative coordinates of the canonical axis 2. This specimens group is at the intersection of scatted plot formed by *R. B. annulatus* and *R. B. microplus*.

The Mahalanobis distance estimated between the different studied populations was demonstrated using the Ward aggregation method (Table 5). The largest distance (15.26) separates *R. B. annulatus* specimens from those of *R. B. microplus*. In addition, the distance between hybrids and *R. B. microplus* populations (6.01) was also relatively low. The lowest distance was obtained between hybrids and *R. B. annulatus* populations (4.23). All these distances between these populations are statistically significant (p <0.001).

The dendrogram resulting from the ascending hierarchical classification revealed 3 population groups at aggregation distance 50 (Figure 4). Group I consists of *R. B. annulatus* population while group II belongs to *R. B. microplus* specimens group and group III consists solely of hybrid population. On contrary, each of these population groups is constituted of different subgroups at the segregation distance 10.

**Table 5: Distances of Mahalanobis between the 3 populations studied.**

<table>
<thead>
<tr>
<th></th>
<th><em>R. B. microplus</em></th>
<th><em>R. B. annulatus</em></th>
<th>hybrid</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. B. microplus</em></td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>R. B. annulatus</em></td>
<td>15.26</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>hybrid</td>
<td>6.01</td>
<td>4.23</td>
<td>0.00</td>
</tr>
</tbody>
</table>
DISCUSSION

Two findings were made during this study. The first concerns the existence of male \( RB_{microplus} \) and female \( RB_{annulatus} \) couples on farms selected for this study. This finding confirms the suspicion of possible natural mating between \( RB_{microplus} \) and \( RB_{annulatus} \) (Graham et al., 1966). Also, the exclusive observation of male \( RB_{microplus} \) and female \( RB_{annulatus} \) couples may be due to the fact that male ticks of the \( RB_{microplus} \) species are sexually more active than the male ticks of the \( RB_{annulatus} \) species. This finding confirms Davey et al. (1983) work which has demonstrated that male \( RB_{microplus} \) ticks were more active for mating than \( RB_{annulatus} \) males.

In addition, the exclusive observation of couples indicates, on the hand, the efficiency of microplus males in finding sexual partners and, on the other hand, attests to the numerical importance of female \( annulatus \) on farms. Indeed, the tick \( RB_{microplus} \) was reported for the first time in the north of the country in 2012 (Kouassi, 2012). However, its introduction into this area where \( RB_{annulatus} \) ranked 2nd on abundance importance was estimated between 2009 and 2011 (Gragnon et al., 2018). This kind of environment is suitable for interspecific crosses between \( RB_{microplus} \) males and \( RB_{annulatus} \) females.

On the other hand, the observation of hybrid ticks of female sex fixed on animals confirms the natural mating of these 2 species and the existence of viable descents. These results confirm those obtained by Graham et al. (1983) and Thompson et al. (1980) during their crossbreeding tests between \( RB_{microplus} \) and \( RB_{annulatus} \) in laboratory. The almost exclusive presence of female hybrid ticks may be due to the fact that hybrid male larvae may be more sensitive to high temperatures and low hygrometry than females (1983) and secondly, by the fact that the parasitic period of male hybrids is shorter 46.6 days (ranging from 28 to 73 days) whereas it is 42 days for males \( RB_{microplus} \) pure strain (Thompson et al., 1980).

**Intragroup morphometric variability**

The results of univariate and multivariate statistics performed on the metric descriptors selected in this study showed very remarkable morphometric differences between the three populations of \( RB \) ticks studied. Elementary statistics revealed high coefficients of variation, ranging from 16.10 to 33.00%, for almost all variables measured in all the 3 tick populations (CV >15%). All tick populations investigated were heterogeneous for all the metric characters studied. The phenotypic heterogeneity observed within those Populations seems to be the consequence of an individual adaptation of the different specimens to the environmental conditions of this area. This hypothesis is supported by Sardi and al. (1998) who argue that phenotypic heterogeneity within a fish population is an adaptive response of some individuals to environmental variation. This intra-populational phenotypic plasticity is the result of an interaction between environmental and genetic factors (Murta, 2000).

All herds sampled regularly use acaricides to control tick infestation. The frequent application of different types of these products, often under-dosed, constitutes multiple aggressions that surviving ticks must cope with. As a result, ticks constantly face constant environmental variations.

**Intergroup morphometric differentiation**

Multivariate analysis showed that all morphometric characters measured particularity Lt, Lca, Lop and Lpa showed highly significant difference between the three species. According to these morphometrics characters, the projection on canonical axis, showed clearly distinct
specimens groups of *R B annulatus* and *R B microplus* populations. Polygons representing hybrid population are an intermediate group between *R B* strains groups. The Mahalanobis distance estimated between the studied populations showed that hybrids are slightly greater proximity to *RB annulatus* than *R B microplus*.

These results show a morphological proximity between *RB* hybrids and *R B annulatus*. In addition, the ascending classification based on the most discriminating morphometric characters revealed for each of these groups of ticks the existence of subpopulations at the aggregation distance 10. Some of these subgroups of individuals could be due to the fact that they come from the same farms or that they come from backcrossing between hybrid females and males from one of the parent species. The existence of second generation hybrid tick subgroups is more plausible because female hybrid ticks from *R B microplus* and *R B. annulatus* are fertile (Thompson *et al.*, 1981). As a result, their backcrossing with pure parental breed specimens increases their homozygosity and yields specimens that are closest to the parental species morphologically.

**CONCLUSION**

This study of morphological characters of *R B annulatus*, *R B microplus* and one of their hybrids is a preliminary work in this research. The results showed that the metric characters allowed us to differentiate species of RB. Hibrad ticks populations were morphologically different from *R B annulatus* and *R B microplus* although the closeness between female hybrids and *R B annulatus* females is greater.

Further study of the genetic differentiation of individuals of different species is needed to confirm the results of this study. Genetic analyzes would reveal whether *R B microplus* and *R B annulatus* differentiate easily and if there is no 2nd generation hybrid, there is retrogression between the hybrid females observed in this study and male ticks belonging to one of the pure parenting species or grandparenting.

**REFERENCES**


