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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* genuses were taken from cattle farms located in northen 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 Boophylus*, *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 quickly 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 *Boophylus 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 (Lt), 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:

Title	***R B annulatus***		***hybrids***		***R B microplus***	
	Mean ± sd	**cv**	**Mean ± sd**	**cv**	**Mean ± sd**	**Cv**
Lt	5.468±1.330	24.32%	3.990±0.643	16.10%	2.293±0.454	19.80%
L. CA	1.014±0.239	23.58%	1.171±0.223	19.07%	0.574±0.189	33.00%
L. POD	2.276±0.710	31.18%	1.236±0.270	21.88%	0.888±0.188	21.13%
L. OP	2.184±0.703	32.20%	1.576±0.432	27.41%	0.831±0.262	31.57%
L. PA	1.672±0.347	20.74%	1.558±0.270	17.34%	1.951±0.460	23.57%

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.

	Axis 1	**Axis 2**
Eigenvalues	3.138	0.985
% Total variance	62.773	19.707
Cumul %	62.773	82.480
Lt	-0.989	-0.098
Lca	-0.663	0.211
Lpo	-0.885	-0.193
Lop	-0.936	-0.108
Lpa	0.244	-0.939

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.

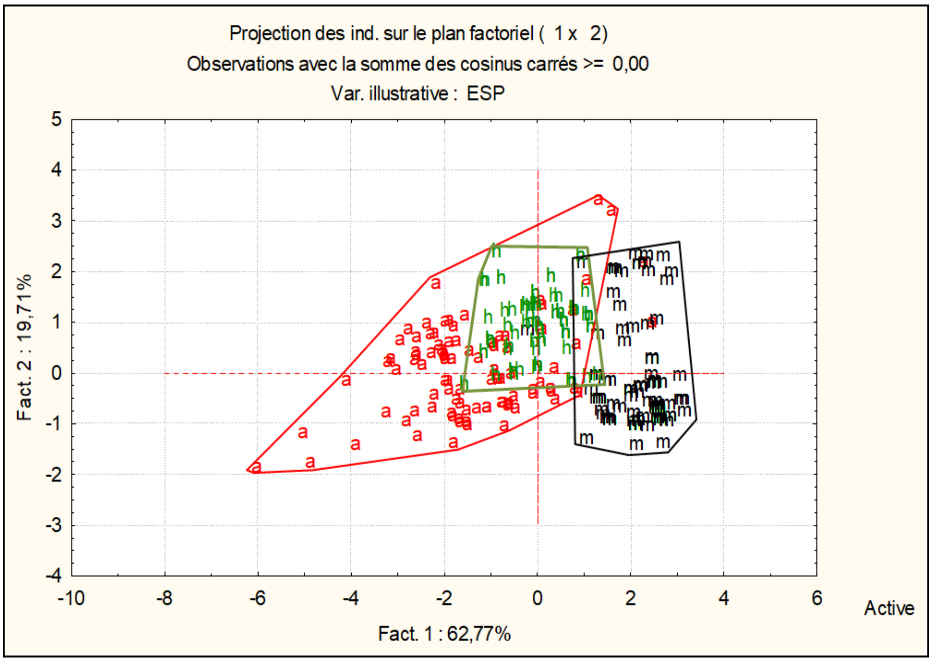

Figure 2: Projection of *tick* populations in the factorial axis 1 and 2 of morphometric parameters' PCA.

The specimens belonging to *R B microplus* group of 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 scatted 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 (λ)], there is the Lop (λ = 0.2240), the Lt (λ = 0.2207), the Lca (λ = 0.2199) and the Lpa (λ = 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.

Variables	Wilk (Lambda)	P values	Toler.
Lt	0.2207	***0.0049***	0.0709
Lca	0.2199	***0.0081***	0.4141
Lpo	0.2141	0.4200	0.1750
Lop	0.2240	***0.0005***	0.2185
Lpa	0.2179	*0.0307*	0.9909

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

Groupes	% Correct	*R B microplus*	*R B annulatus*	hybrid
R B microplus	89.78	123	0	14
R B annulatus	86.04	0	74	12
Hybrid	80.77	4	11	63
Total	86.38	127	85	89

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).

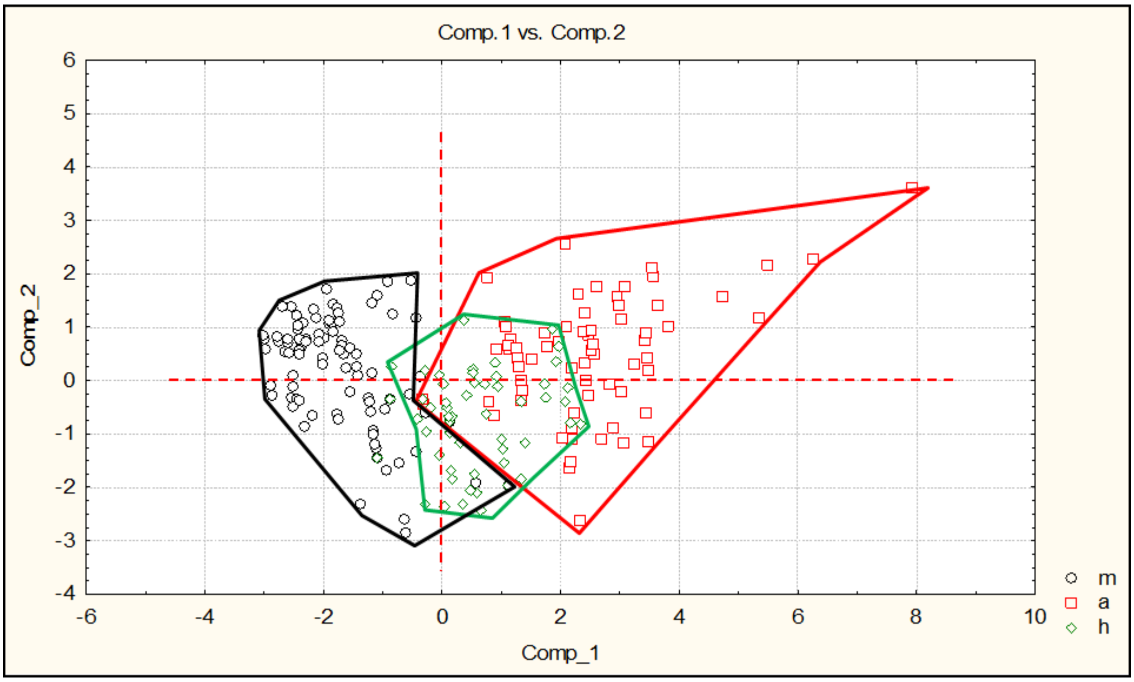

Figure 3: Distribution of the different populations in the DFA canonical plans 1 & 2 according to metric descriptors.

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.

	R B microplus	***R B annulatus***	***hybrid***
R B microplus	0.00		
R B annulatus	15.26	0.00	
hybrid	6.01	4.23	0.00

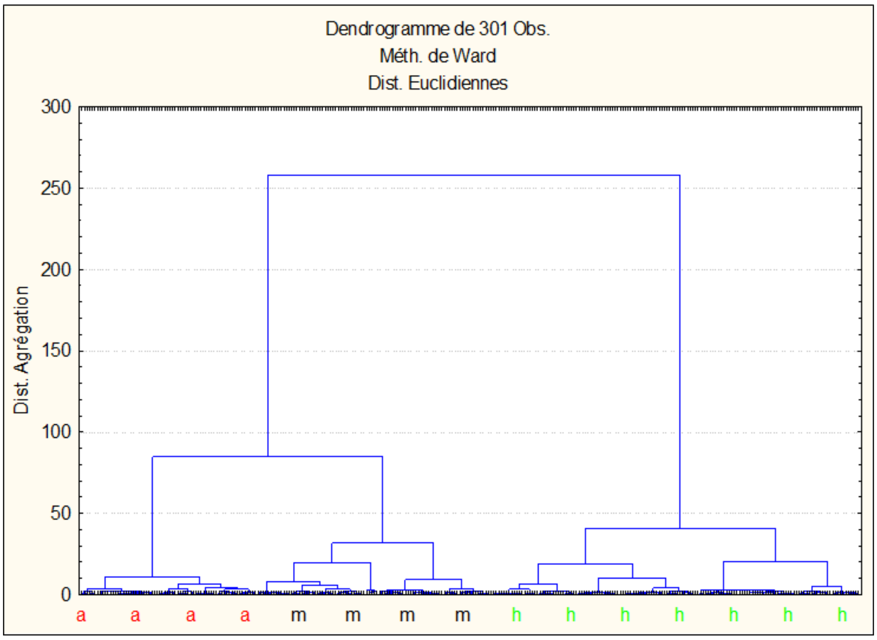

Figure 4: Dendrogram of 3 populations according to Ward's method.

DISCUSSION

Two findings were made during this study. The first concerns the existence of male *R B microplus* and *female R B annulatus* coupled on farms selected for this study. This finding confirms the suspicion of possible natural mating between *R B microplus* and *R B annulatus* (Graham *et al*., 1966). Also, the exclusive observation of male *R B microplus* and female *R B annulatus* couples may be due to the fact that male ticks of the *R B microplus* species are sexually more active than the male ticks of the *R B annulatus* species. This finding confirms Davey *et al*. (1983) works which have demonstrated that male *R B microplus* ticks were more active for mating than *R B 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 *R B microplus* was reported for the first time in the north of the country in 2012 (Kouassi, 2012). However, its introduction into this area where *R B 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 *R B microplus* males and *R B 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 *R B 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 *R B* ticks studieds 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 Sardà *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 acaricids 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 particulary 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 *R B* 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. Hibrid 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.

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