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Original Research Article

Intraspecific variation in *Rhipicephalus* (*Boophilus*) *microplus* ticks (Family *Ixodidae*) isolated from goats raised in a small private farm in San Jose Del Monte, Bulacan, Central Luzon, Philippines

Priscilla Hope Swann*, Florencia G. Claveria

Biology Department, College of Sciences, De La Salle University, 2401 Taft Avenue, Manila Philippines

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**Corresponding author email:*
swann.hope@gmail.com

Abstract

This study was carried out to ascertain tick infestation of goats (*Capra aegagrus hircus)* grown in a small private farm in San Jose del Monte, Bulacan, Central Luzon, Philippines. Prevalence of infestation was assessed and ticks examined based on their developmental stages and sex were identified as *Rhipicephalus* (*Boophilus*) *microplus.* Adult ticks displayed intraspecific polymorphic variations (ISPV). Male *Rh.* (Bo.) *microplus* manifested variations in the distinctness of the ventral anal plate spurs (88.2%), and in the visibility of these same spurs when specimens were viewed dorsally. ISPV was detected in the shape or contour of the genital aperture lips in 37.5% of female ticks. To our knowledge, this study represents the first documentation of *Rh.* (*Boophilus*) *microplus* infestation in Philippine goats vis-à-vis demonstration of intraspecific polymorphic variations between male and female individual ticks.

Keywords: *Rhipicephalus* (*Boophilus*) *microplus*, Ticks, Intraspecific polymorphic variation, Goats, Philippines

Introduction

Ticks are hematophagous ectoparasitic arthropods and can transmit deadly viral, bacterial, and parasitic diseases. Worldwide, *Rhipicephalus* (*Boophilus*) spp., *Ixodes* spp., and *Hyalomma* spp. infest livestock and domestic animals (Mehlhorn, 2008). In Asia, infestation of goats and sheep with *Rhipicephalus* (*Boophilus*) spp., *Ixodes* spp., and *Hyalomma* spp., as well as *Haemaphysalis* spp., *Amblyomma* spp. and *Dermacentor* spp. have been reported (Daemon et al., 1998; De Waal, 2000; Yukari and Umur, 2002; Bock et al., 2004; Al-Khalifa et al., 2007; Hove et al., 2008; De Matos et al., 2009), together with tick-borne blood parasites, *Babesia*, *Anaplasma*, and/or *Theileria* (Petney, 1993; Jittapalapong et al., 2005; Al-Khalifa et al., 2007). These blood parasites can lead to anemia and cerebral derangement (Ahmadi-Hamedani et al., 2009; Sulaiman et al., 2010), high morbidity and mortality (Irshad et al., 2010) in animals, and can also be harmful to consumers of milk products processed from infected animals (Holzmann et al., 2009).

In the Philippines, there are documented evidence of the presence of tick-born protozoan parasites in cattle and water buffaloes with *Babesia* spp. and *A. marginale* (Dumag and Reyes, 1960; Molina and Montenegro, 1977; Padilla et al., 2006; Foronda et al., 2010); horses with *Babesia* parasites confirmed through detection of anti-*B. caballi* and/or anti-*B. equi* antibodies using immunochromatographic test (ICT) (Cruz-Flores et al., 2010), and polymerase chain reaction (Yu et al., 2013). The use of ICT has also

revealed the presence of anti-*B. gibsoni* antibodies in *Rhipicephalus* ticks-infested dogs (Cruz-Flores et al., 2008).

Due to developments in molecular biology technology, the taxonomy of ixodid ticks, previously organized as per their morphological characters, has undergone re-organization. Murrell and Barker (2003) recommended that *Boophilus* be synonymized with *Rhipicephalus* based on previous molecular studies done using mitochondrial DNA, 12s rRNA, 16s rRNA, and tRNA (Barker and Murrell, 2004). The use of polymerase chain reaction is thought to be a more reliable method of identifying tick species due to the presence of naturally-occurring intraspecific polymorphic variations that could be misleading (Abdigoudarzi et al., 2011). However, recent studies have used the identification of morphological and biological intraspecific variations in concert with identified genetic intraspecific variations to determine that *Rhipicephalus* spp. may need to be re-described as some of these may have developed species divergences (Szabó et al., 2005; Dantas-Torres et al., 2013). In this paper, we documented findings of intraspecific polymorphic variations in adult, male and female, *Rhipicephalus* (*Boophilus*) *microplus* ticks infesting goats in a private farm in Luzon, Philippines.

Materials and Methods

Study area and tick collection and preservation

Goats examined were raised in a private farm in San Jose del Monte in Bulacan, Central Luzon, Philippines. The single herd had 18 goats initially (13 females; 5 males) aged two months to six years. This herd had unrestricted movement in the farm and were neither de-ticked nor given any medications or vitamins. With the help of a caretaker, ticks were collected, within three months, from the head, ears, neck, belly, back, legs, perineum, and tail of each goat, and the number of ticks collected per body part was recorded. To prevent the legs from curling and obscuring tick abdomens, a process essential in species identification, ticks plucked per goat were initially fixed in Boardman's solution (17% EtOH + 3% ether) for 24 hours, and transferred to 80% EtOH + 5% glycerol, prior and their examination using Nikon Eclipse E400 Stereoscopic Microscope in Parasitology Laboratory, Science Technology Research Center, De La Salle University, Philippines

Ascertainment of developmental stages of collected ticks

Using Walker et al. (2007), as a reference, tick developmental stages were ascertained as follows: larva (with six legs); nymph (with eight legs without a genital aperture, and adult (with eight legs and a genital aperture), and stages were segregated. The identification of species was anchored on the information taken from Walker et al., 2007, as well as a dichotomous key (Sonenshine and Roe, 2013), on the sex-specific morphological characters of male and female ticks. Individual male and female ticks were examined using Nikon Eclipse E400 Stereoscopic Microscope (Parasitology Laboratory, Science Technology Research Center, De La Salle University for any manifestations of intraspecific polymorphic variations (ISPV). Discrepancies in these characteristics were appropriately documented.

Data Analysis

The existence of sex-specific features was recorded individually in 30 each of adult male and female ticks and occurrence of intraspecific polymorphic variation was compared (Walker et al., 2007), and any variations were recorded. Micrographs and, when necessary, line drawings were prepared for better characterization of the variations.

Results

Taxonomic Classification of Tick Specimens

The ticks were identified as ixodids (Family Ixodidae) belonging to genus *Rhipicephalus* (*Boophilus*) (Sonenshine and Roe, 2013). Based on adult morphological sex-specific characteristics (Walker et al., 2007), the species was identified as *Rhipicephalus* (*Boophilus*) *microplus*.

Female ticks possessed broadly oval porose areas on the dorsal region of the basis capitulum (Fig. 1A); a genital pore or aperture with a posterior lip shaped like a broad "u" (Fig. 1B); and two distinct spurs on the first coxae, and an obvious cleft between them, as well as smaller spurs on the second coxae (Fig. 1C).

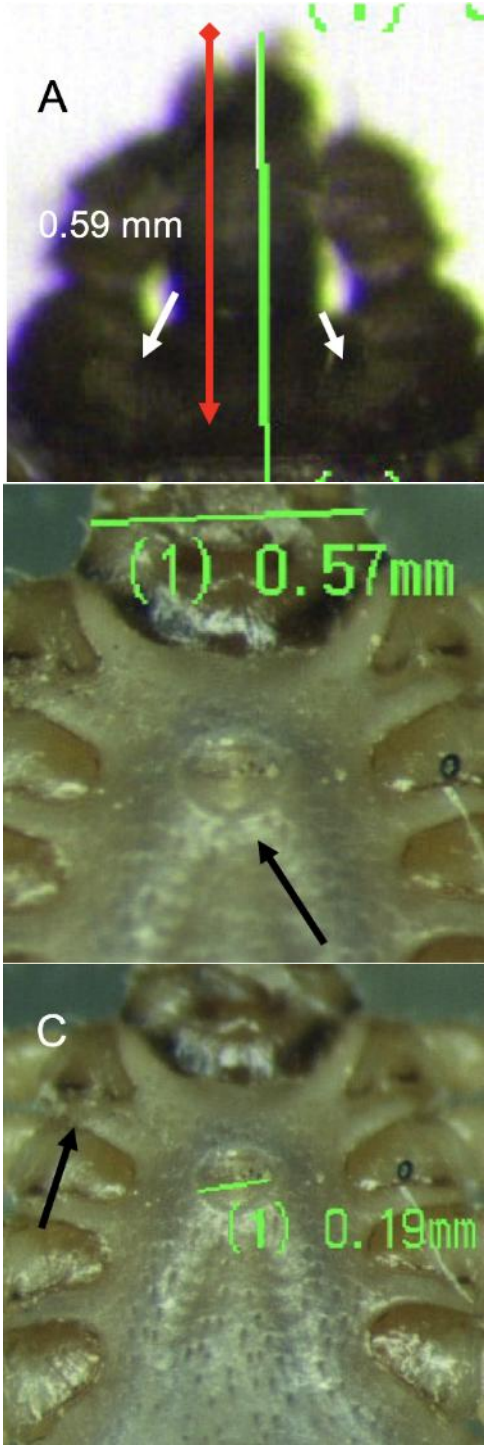

Figure 1: Female *Rh.* (*Bo.*) *microplus*. A: Dorsal view photomicrograph with broad oval porose areas (indicated with arrows). B: Ventral view, genital aperture with posterior lip shaped like a broad "u" (arrow). C: Ventral view, spurs on coxae 1 to 3. Spurs on coxa 1 have a distinct cleft between them (arrow).

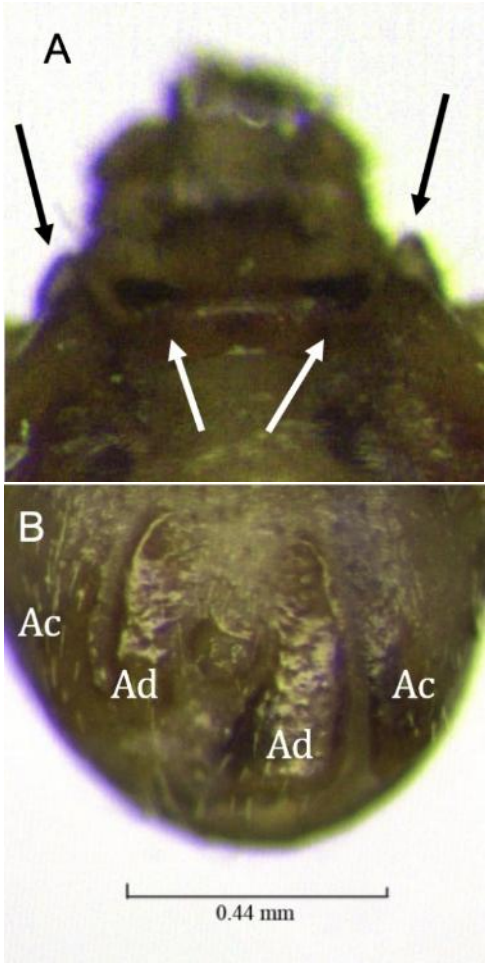

Figure 2: Male *Rh.* (*Bo.*) *microplus*. A: Dorsal view photomicrograph with cornua (white arrows) and anterior spurs (black arrows). B: Ventral view, indistinct anal plates (Ad: adanal; Ac: accessory).

Male ticks exhibited cornua, horn-like protrusions from the dorsal posterior region of their basis capituli and spurs positioned anteriorly to coxae 1 reminiscent of shoulder pads (Fig. 2A); with paired ventral accessory and adanal anal plates with indistinct spurs, which were not visible dorsally (Fig. 2B).

Intraspecific polymorphic variation in *Rh.* (*Boophilus*) *microplus*

The 30 female *Rh.* (*Bo.*) *microplus* ticks were examined for variations in four sex-specific traits, namely: I. the presence of paired broad oval porose areas on the dorsal surface of the basis capitulum; II. distinct, paired coxae 1 spurs with an obvious cleft between them; III. coxae 2 and 3 spurs; and IV. broad

"u"-shaped genital aperture posterior lips (GAPL) (Table 1). Photomicrographs of broad "u"-shaped GAPL (Fig. 1A) were difficult to capture, as the posterior lips were less bulbous than those of "v"-shaped GAPL.

All 30 female adult ticks manifested Traits II and III; 24 manifested Trait I, with the remaining 23.3% unconfirmed for that trait. This was due to damage to the tick capituli during specimen preparation and to obstructions owing to the presence of leftover cement, secretions in the saliva that help the mouthparts attach to the host, or animal host matter such as skin and hair. Trait IV was observed in 21 specimen, while 10% were unconfirmed due to damage to the GAPL area during specimen preparation.

Table 1: Comparison of 30 female *Rh.* (*Bo.*) *microplus* manifesting sex-specific traits. n = 30

	I. (%)	II. (%)	III. (%)	IV. (%)
Present	23 (76.7)	30 (100)	30 (100)	21 (70)
Absent	0	0	0	6 (20)
Unconfirmed	7 (23.3)	0	0	3 (10)

Interestingly, six female manifested variations in the shape of the GAPL: four manifested a broad "v"-shaped genital aperture posterior lips (Fig. 3B), and the two had narrow "v"-shaped genital aperture posterior lips (Fig. 3C). Width of the GAPL measured: 0.12 – 0.36 mm (average 0.17 mm) for broad "u"-shaped GAPL, 0.15 – 0.22 mm (average: 0.19 mm) for narrow "v"-shaped GAPL, and 0.17 – 0.22 mm (average: 0.19 mm) for the broad "v"-shaped GAPL.

Seventeen male *Rh.* (*Bo.*) *microplus* ticks were examined for variations in four sex-specific traits, namely: I. distinct anterior coxae 1 spurs; II. distinct cornua at the base of the dorsal portion of their capitulum; and III. indistinct ventral anal plate spurs (VAPS), which are IV. not visible dorsally. All 17 males possessed Traits I and II (Table 2). However, a striking variation was noted in Traits III and IV. Fifteen males had distinct VAPS (Fig. 4B), compared to the single specimen with indistinct VAPS. Of the 15 males with distinct VAPS, there were seven ticks with spurs non-visible dorsally, and six visible dorsally (Fig. 4D). The remaining three ticks were unconfirmed for trait IV, as the dorsal body of the specimens had been damaged during preparation. The adanal plates were measured along their diagonal; these plates had an average diagonal length of 0.29 – 0.67 mm (average: 0.51mm). The accessory plates were measured from root to spur: 0.25 – 0.49 mm (average: 0.36 mm).

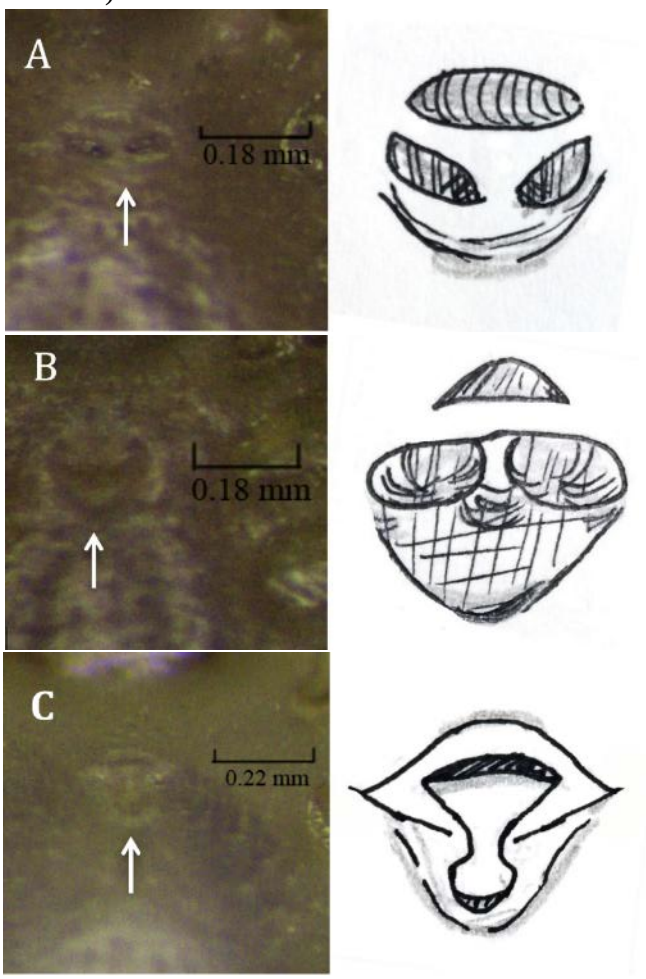

Figure 3: Photomicrograph and line drawings of female *Rh.* (*Bo.*) *microplus* genital apertures and genital aperture posterior lip (GAPL). A: Broad "u"-shaped GAPL (arrow). B: Broad "v"-shaped GAPL (arrow). C: Narrow "v"-shaped GAPL (arrow).

Table 2: Comparison of 17 male *Rh.* (*Bo.*) *microplus* manifesting sex-specific traits.

	I. (%)	II. (%)	III. (%)	IV. (%)
Present	17 (100)	17 (100)	1 (5.9)	7 (43.8)
Absent	0	0	15 (88.2)	6 (37.5)
Unconfirmed	0	0	1 (5.9)	3 (18.8)

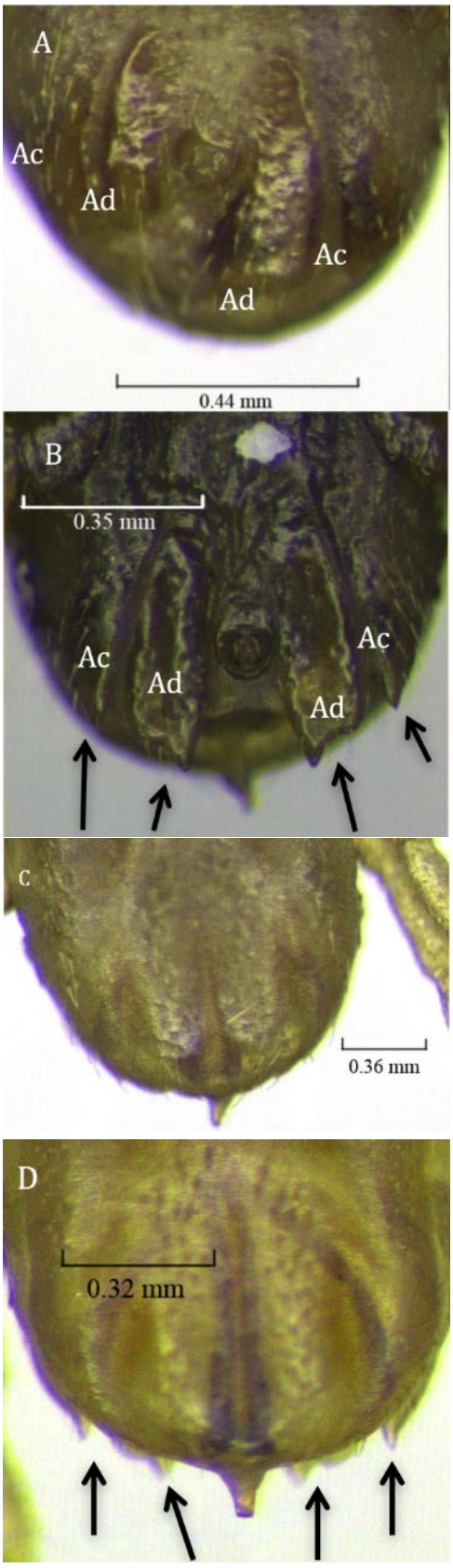

Figure 4: Male Rh. (Bo.) microplus. A: Ventral view, anal plates with indistinct accessory (Ac) and adanal (Ad) ventral anal plate spurs. B: Ventral view, distinct accessory (Ac) and adanal (Ad) ventral anal plate spurs (arrow). C: Dorsal view, ventral plate spurs are not visible, D: Dorsal view, ventral plate spurs (arrow) protrude beyond the margin of the conscutum.

Discussion

A small host population would most likely lead to high levels of inbreeding and homogeneity in tick species populations. In the present study however, intraspecific polymorphic variation was apparent in sex-specific characteristics of *Rh.* (*Bo.*) *microplus* specimens, such as the distinctness and dorsal visibility of male ventral anal plate spurs, and the shape of female genital aperture posterior lips. Intraspecific polymorphic variations (ISVs) occur as well, in other *Rhipicephalus* spp. (Szabó et al., 2005), and Dantas-Torres et al. (2013) maintain that ISVs are probably a naturally occurring process, especially when working with widely distributed tick species like *Rh.* (*Bo.*) *microplus.*

Hybrids of *Rh.* (*Bo.*) *decoloratus* and *Rh.* (*Bo.*) *microplus* (Nyangiwe et al., 2013), and between *Rh.* (*Bo.*) *annulatus* and *Rh.* (*Bo.*) *microplus* (Hilburn et al., 1991) have manifestations of ISVs. Abdigoudarzi et al. (2011) argue that the identification of ixodids based on morphological characters can be intricate by the appearance of ISVs resulting from post-feeding and factors related to tick preservation. Increasing the amount of ISV data is valuable in resolving questions related to the proposed changes in the taxonomy of rhipicephaline ticks, alongside molecular evidence that led to the discovery of the synonymy of *Boophilus* and *Rhipicephalus* (Beati and Keirans, 2001; Murrell and Barker, 2003; Barker and Murrell, 2004). In view of all these morphological and molecular data, the documentation of ISPs in an essential input in redefining the taxonomy and systematics, particularly of the rhipicephaline group of ixodids,

In conclusion, this report of infestation of goats with *Rh.* (*Bo.*) *microplus* manifesting intraspecific polymorphic variation in adult male and female ticks represents the first documented case in the Philippines. Future studies should cover larger goat, and other ruminant, populations in different locations/regions and across a longer collection period. Additionally, the researchers should investigate whether cross-infection

between cohabiting domesticated ruminants exists. These studies should be a combination of biological, morphological, and molecular approaches so as to ensure the proper identification of any tick species that may be collected.

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