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Molecular detection of *Rhipicephalus* (*Boophilus*) *microplus* and *Haemaphysalis* species infesting cattle from different agro climatic zones of Mizoram

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Abstract
The present study describes morphological and molecular detection of *Rhipicephalus* (*Boophilus*) *microplus and Haemaphysalis* spp. Infesting cattle from different agro climatic zones of Mizoram. Microscopy study of *Rhipicephalus* (*Boophilus*) *microplus* revealed hexagonal basis capitulum in both sexes, shorter mouth parts and absence of festoons on the posterior border. Morphologically, the mouthparts of *Haemaphysalis* spp. showed laterally projected conical palps. They were without any coloration and there were no eyes but presence of festoons on the posterior regions. The morphological features of both genera were further confirmed by polymerase chain reaction (PCR) which selectively amplified a fragment length of about 400 bp for *Rhipicephalus* (*Boophilus*) *microplus* and about 376 bp for *Haemaphysalis* species. It seems that cattle can be infested by both species in Mizoram.

Keywords: *Rhipicephalus* (*Boophilus*) *microplus*, *haemaphysalis*, cattle, LM study, PCR, Mizoram, India

1. Introduction
Various species of ticks are often found in cattle but the intensity of infestation and the effects of ticks on their hosts are poorly studied [1]. The bovine tick *Rhipicephalus* (B.) *microplus* can be found in multiple tropical and subtropical regions worldwide [2]. Although the history of the dissemination of *R.* (*B.*) *microplus* is not well documented, the species is known to have originated in India [3]. As per literature, *R.* (*B.*) *microplus* originated in the southern and south eastern regions of Asia and was spread throughout the tropical and subtropical belts via cattle. One tick genus of recent importance is *Haemaphysalis*. Native to East Asia, various species of this genus have become invasive in multiple regions of the world, largely due to its parthenogenetic reproduction, broad habitat use, and high diversity of avian and mammalian hosts [4, 5, 6].
One of the key morphologic characteristics used to differentiate ixodid ticks are the mouthparts but often these features are damaged during tick collection, making species identification difficult or impossible [7, 8]. The natural history and spread of these two tick species rely on the quick and accurate identification of ticks using key morphological features found on the mouth parts. However, this is difficult if the specimen's mouthparts are damaged during removal from a host. In these cases, molecular confirmation is needed to identify the ticks to the species level.
The aim of this study is to develop a molecular assay to quickly identify various species of *Rhipicephalus* (*B*) *and Haemaphysalis*.

2 Materials and methods
2.1 Collection and preservation of ticks
Ticks were obtained from different agroclimatic zones of Mizoram (Figure 1, Table 1). Ticks were morphologically identified as per keys [9]. All ticks were stored in 70–100% ethanol and morphological identification was done with dissecting and compound light microscopy using dichotomous keys to distinguish between the species when possible.

2.2 DNA extraction and PCR amplification

DNA was extracted from both genera of ticks by using a commercial kit (DNeasy® Blood and Tissue kit, Qiagen) following manufacturer's protocol with slight modification. Briefly, one female tick of each genus was triturated with buffer and Proteinase K was added to the mixture and incubated at 56°C for complete lysis. After adding ethanol (96-100%) the mixture was transferred into a spin column and centrifuged. After washing with buffer twice, the elution buffer was added to the column membrane and incubated for 2 min at room temperature. Finally the column was centrifuged at filtrate was collected and kept in -20 °C. Polymerase chain reaction (PCR) was done following the standard time-temperature protocol using the oligonucleotide primers, F – 5' AAA CTA GGA TTA GAT ACC CT 3' and R – 5' AAT GAG AGC GAC GGG CGA TGT 3' to amplify the mitochondrial 16S rRNA gene fragment of each genus.

3. Results

3.1 Male *Rhipicephalus* (*B*) *microplus*

Males were found to be much smaller than female (Figure 2). Hypostome is toothed with dental formula of 4/4. Basis capituli is hexagonal dorsally. The whole dorsal surface is covered with scutum. Coxa I bears a median external spur and a short internal spur. The ventral surface is with accessory adanal ventral plates and the posterior part posses an appendage without any festoons.

3.2 Female *Rhipicephalus* (*B*) *microplus*

Both dorsal and ventral surface of the female tick was examined microscopically. The position of basis capitulum is sub-dorsal and the posterior border is found devoid of any festoons. Left palp showed two teeth like protuberances and the right one showed one similar structure. The dorsal surface of basis capitulum bears two porose areas which are pyriform in shape. The scutum covers one third of the total body surface (Fig 3).

3.3 Male *Haemaphysalis* spp.

The body is oval shape. Scapulae are short and blunt; the margins are narrow and shallow; cervical grooves are deep, linear anteriorly and converging posteriorly; lateral grooves are narrow and long, enclosing the first two anterior festoons; eyes absent; 11 festoons. Legs were short and robust. All coxae have a well-developed inner spur, similar in size and shape.

3.4 Female *Haemaphysalis* spp.

Scutum lacks ornamentation, having a dark brown color and the body is elliptical-shaped. Basis capituli is about 3 times wider than long; cornua absent. The porose areas are moderate size, oval, and distantly spaced, with a shallow depression between them. Hypostome is slightly shorter than the palpi (Fig 3).

An approximately 400bp of the ITS2 region of 16s mitochondrial rRNA of *Rhipicephalus* (*Boophilus*) was amplified from the collected tick for molecular confirmation. Genomic DNA was amplified and the amplification of specific PCR product was checked by gel electrophoresis with 1.5% agarose gel and viewed in UV trans illuminator system (Figure 4).

An approximately 376bp of the ITS2 region of 16s mitochondrial rRNA of *Haemaphysalis* was amplified from the collected tick for molecular confirmation. Genomic DNA was amplified and the amplification of specific PCR product was checked by gel electrophoresis with 1.5% agarose gel and viewed in UV trans illuminator system (Fig 4).

Table 1: Geographic and characteristic features of Agro climatic zones in Mizoram

Sl. no	Agro-Climatic Zone & Altitude Range (amsl)	Average Rainfall (mm/yr)	Mean Temperature (Max-Min)	Area (Km^2)	Study Villages Selected
1	Humid Mild Tropical Hill Zone (less 800 m)	2000-3000	30 - 12 °C	14,733 (69.87%)	Zawlnuam, West Phaileng, Mamit, Phuldungsei
2	Humid Mild Sub-Tropical Hill Zone (800-1400 m)	2500-3000	30 - 12 °C	5581 (26.47%)	Muthi, Thingsul, Sihphir
3	Humid Temperate Sub-Alpine Zone (1400 m or more)	2000-3000	20 - 11 °C	773 (3.67%)	Khawzawl, Hnahthial, Vangtlang.

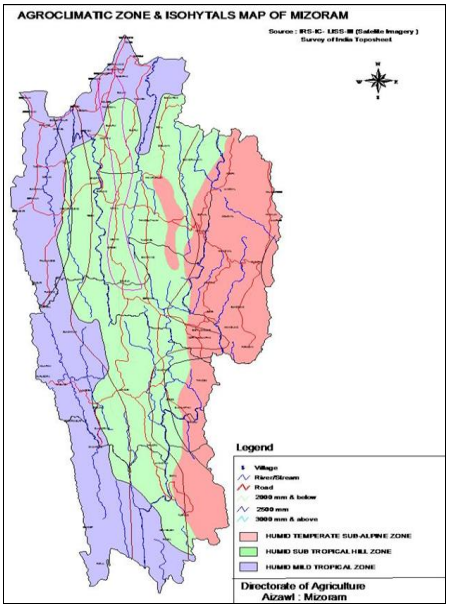


Fig 1: Demarcation of different agro-climatic zones of Mizoram

Fig 2: Male *Rhiphicephalus* (*B*) *microplus*

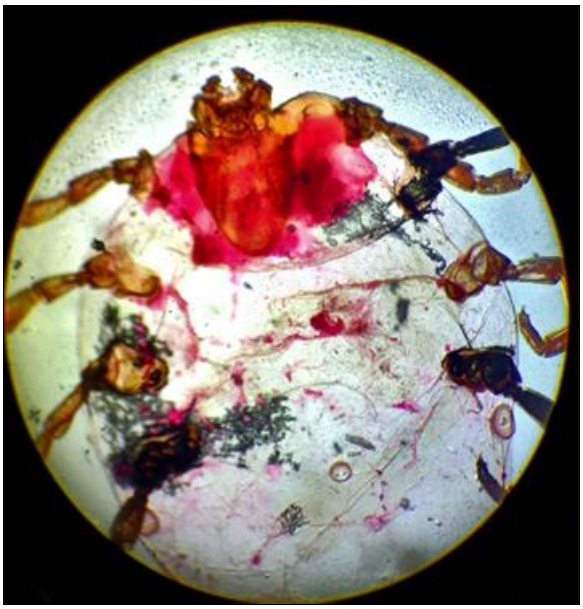
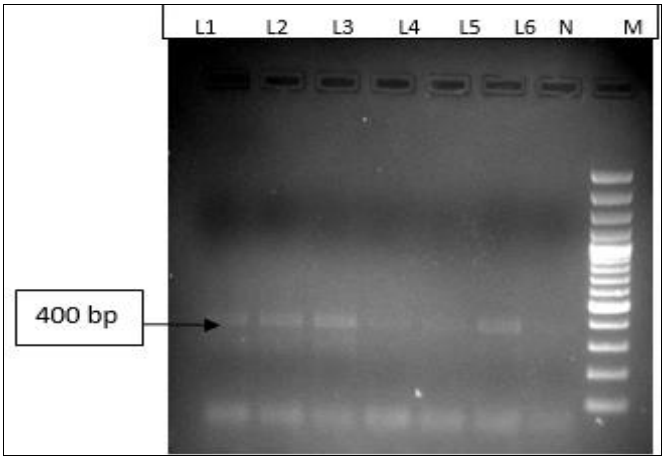


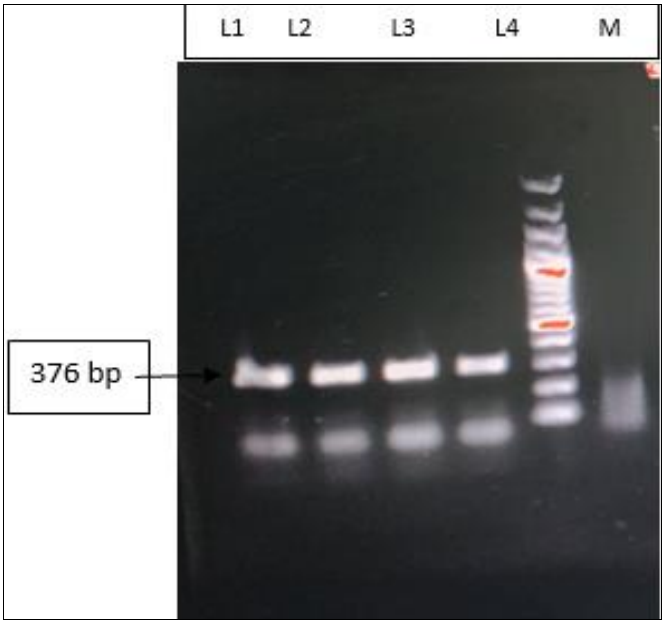
Fig 3: Female *Rhiphilus* (*B*) *microplus*

Fig 4: anterior part of female *Haemaphysalis* spp showing prominent conical palp



L1-L6- Positive amplification
N- Negative
M- Molecular marker

Fig 5: PCR amplification of ITS2 region of 16s mitochondrial rRNA gene fragment (400 bp) of *Rhipicephalus* (*B*) *microplus*



L1, L2, L3, L4: Positive amplification from ticks
M: DNA ladder
M – 100bp plus DNA ladder.

Fig 6: PCR amplification of12S rRNA gene fragment (~ 376bp) of *Haemaphysalis spp.* in 1.5% agarose gel. L1 – L4 – Positive amplification

4. Discussion

This tick survey that took place in different agro climatic zones of Mizoram provides the first through survey of Ixodid ticks in this state and provides the morphological and molecular evidence for the presence of *Rh.* (*B.*) *microplus* and *Haemaphysalis* species. The invasive characteristic of *Rh.* (*B.*) *microplus and Haemaphysalis* has been attributed to many factors including higher reproductive output combined with a shorter generation length, (especially in humid and tropical forest habitat), and to the acaricide resistance acquired by this tick species to most of the acaricides available on the market [10]. Considering the vast suitable environmental conditions in mizoram and the preponderance of cattle among the poor rural farmers, the introduced tick, *Rh.* (*B.*) *microplus* and *Haemaphysalis* spp. could become a major threat to livestock production. The conventional method to identify tick specimen relies mostly on morphological characters and

ecological distributions. Species determination using conventional methods (morphology–ecology) are limited for morphologically similar taxa, damaged specimens, and where immature stages are not described or are engorged. This method is cumbersome and difficult since ticks may vary marginally in size and in morphological characters. This can be overcome by using ultrastructural observation of external features and molecular markers such as ITS and 16S rDNA which are highly conserved and easy to amplify using polymerase chain reaction (PCR).

The most distinct feature between the two genera is the basis capitulum, hexagonal in Rhipicephaline tick and rectangular in Haemaphysaline tick which is not visible with naked eyes. Hence, genetic characterization is important to identify the ticks infesting animals. Molecular markers like mitochondrial 12S/16S rDNA, cytochrome oxidase subunit I (COI) and nuclear ribosomal ITS2 have been successfully used to study the evolution and phylogenies of mites and ticks.

The implication of this finding is that there may be additional economic burden to livestock farmers due to increased cost of tick control occasioned by the acaricide resistance by this tick species widely reported from different climates. Additionally, there may be a potential upsurge in incidence of hemoparasitic infections in cattle leading to increased morbidity, cost of treatment and mortalities in mizoram

5. Conflict of interest statement

We declare that we have no conflict of interest.

6. Acknowledgements

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7. References

1. Zug GR, Vitt LJ, Caldwell JP. Herpetology. An introductory Biology of Amphibians and Reptiles 2nd edition. San Diego, Academic Press; c2001.
2. Estrada-Peña A, Bouattour A, Camicas JL, Guglielmone A, Horak I, Jongejan F, *et al*. The known distribution and ecological preferences of the tick subgenus Boophilus (Acari: Ixodidae) in Africa and Latin America. Exp Appl Acarol. 2006;38(2-3):219-235. http://dx.doi.org/10.1007/s10493-006-0003-5. PMid:16596355.
3. Barré N, Uilenberg G. Spread of parasites transported with their hosts: case study of two species of cattle tick. Rev Sci Tech. 2010;29(1):149-160, 135-147. PMid:20617654
4. Hoogstraal H, Roberts FHS, Kohls TGM, Tipton VJ. Review of *Haemaphysalis* (*Kaiseriana*) *longicornis* Neumann (Resurrected) of Australia, New Zealand, New Caledonia, Fiji, Japan, Korea, and Northeastern China and USSR. and Its Parthenogenetic and Bisexual Populations (Ixodoidea, Ixodidae). J Parasitol. 1968;54:1197-21.
5. Heath ACG. Biology, ecology and distribution of the tick, *Haemaphysalis longicornis* Neumann (Acari: Ixodidae) in New Zealand. N Z Vet J. 2016;64:10-20. doi: 10.1080/00480169.2015.1035769
6. Beard C Ben, Occi J, Bonilla DL, Egizi AM, Fonseca DM, Mertins JW, *et al*. Multistate infestation with the exotic disease – vector tick *Haemaphysalis longicornis* - United States, August 2017 – September 2018. Morbitity Mortal Wkly Rep. 2018;67:1310-3. doi: 10.15585/mmwr.mm6747a3
7. Egizi AM, Robbins RG, Beati L, Nava S, Evans CR, Occi JL, *et al*. A pictorial key to differentiate the recently detected exotic *Haemaphysalis longicornis* Neumann, 1901 (Acari, Ixodidae) from native congeners in North America. Zookeys. 2019;818:117-28. doi: 10.3897/zookeys.818.30448
8. Keirans JE, Litwak TR. Pictorial key to the adults of hard ticks, family Ixodidae (Ixodida: Ixodoidea), east of the Mississippi River. J Med Entomol. 1989;26:435-48. doi: 10.1093/jmedent/26.5.435
9. Barros-Battesti DM, Arzua M, Bechara GH. Carrapatos de importância médico-veterinária da região neotropical: Um guia ilustrado para identificação de espécies. São Paulo: Instituto Butantan; c2006. p. 223.
10. 10 Madder M, Thys E, Achi L, Toure´ A, De Deken R. Rhipicephalus (Boophilus) microplus: a most successful invasive tick species in West-Africa. Exp Appl Acarol. 2011;53:139-145.