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General and Local Morphological Anomalies in *Amblyomma lepidum* (Acari: Ixodidae) and *Rhipicephalus decoloratus* Infesting Cattle in Uganda

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Abstract

Morphological abnormalities in ticks seem to be rare phenomena in nature, and are underreported in Africa. In this article, we describe general and local anomalies in two *Amblyomma lepidum* females and one *Rhipicephalus decoloratus* female collected from cattle in Moroto and Kasese districts, Uganda. One *A. lepidum* specimen displayed metagynander gynandromorphism with the presence of both male and female features in the same organism. The second *A. lepidum* female showed slight asymmetry and lacked a genital aperture. The *R. decoloratus* displayed multiple anomalies that included asymmetry on the right side in association with ectromely, chitinous formations and constrictions on the left side. This article presents the first report of metagynander gynandromorphism, as well as genital aperture absence which is not linked to gynandromorphism, in *A. lepidum* collected from cattle.

Key words: morphological anomaly, tick, cattle, Uganda

For several years, morphological abnormalities have been documented among different body segments, appendages, and other parts, in several species of insects, spiders and ticks (Farnsworth 1988). Recently, these teratological deformities have become interesting for arthropodologists as their possible causes and ecological implications in nature are not known (Buczek 1995, Chitimia-Dobler et al. 2017). Among tick species, they seem to be rare phenomena, only occurring in small percentages even within large sample collections (Kar et al. 2015, Prusinski et al. 2015). Tick anomalies were first described over 100 years ago (Neumann 1899), and have since been reported in species belonging to the genera *Argas*, *Ornithodoros*, *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, and *Rhipicephalus* (Siuda 1981; Taylor et al. 1991a, b; Guglielmone et al. 1999; Labruna et al. 2000; Labruna et al. 2002; Keskin et al. 2012; Kar et al. 2015; Keskin et al. 2016). Gynandromorphism, defined as an individual possessing both male and female phenotypic characters (Eritja 1996), is described and reported for a series of hard tick species (Campana-Rouget 1959a,b; Keskin et al. 2012; Nowak-Chmura 2012; Larson and Paskewitz 2016), and represents the most documented abnormality in ticks (Labruna et al. 2000, Labruna et al. 2002, Keskin et al. 2012, Prusinski et al. 2015, Keskin et al. 2016, Chitimia-Dobler et al. 2017, Chitimia-Dobler and Pfeffer 2017, Muñoz-Leal et al. 2018). In addition, other anomalies such as body asymmetry, atrophy, ectromely, fusion of adanal plates and festoons, have also been reported (Campana-Rouget 1959a, Guglielmone et al. 1999, Dergousoff and Chilton 2007, Kar et al. 2015, Keskin et al. 2016, Chitimia-Dobler et al. 2017).

According to the most widely used classification of tick abnormalities (Campana-Rouget 1959a,b), two general kinds exist: general anomalies, including changes in body shape, asymmetry, nanism, gigantism, gynandromorphism, and duplication, as well as local anomalies such as missing body parts, fusion of adanal plates, malformation of capitulum, abnormalities of appendages and of festoons. Specifically, gynandromorphism in ticks was classified into five types (Campana-Rouget 1959b): 1) bipartite protogynander, where external characters of male and female ticks are equally represented; 2) deuterogynander, where characters of one sex are decreased to a quadrant; 3) metagynander, where characters of one sex are decreased to a small segment; 4) gynander intriqué, a protogynander or deuterogynander where some pieces of male or female characters are embedded in areas of the opposite sex; and 5) mosaic gynandromorphism, where there is no definitive line separating one sex from the other, but pieces are nested without indication of symmetry.

Despite the numerous reports of tick abnormalities in nature, there is only limited knowledge about the occurrence of this phenomenon in sub-Saharan Africa (Dipeolu 1989). Here, we present general and local anomalies in two *Amblyomma lepidum* specimens and multiple anomalies in one *Rhipicephalus decoloratus* female collected from cattle in Moroto and Kasese districts, Uganda.

Materials and Methods

Ticks were collected by handpicking from cattle in five districts of Uganda, i.e., Moroto, Soroti, Gulu, Hoima, and Kasese, from September to November of 2017. Ticks were kept alive in 50-ml plastic tubes until identification in the laboratory at Uganda Virus Research Institute, Entebbe, Uganda. The three ticks with anomalies were identified to species level using morphological characters (Voltzit and Keirans 2003, Walker et al. 2014) under a stereomicroscope (Stereo Discovery V12, Zeiss, Birkerød, Denmark). In addition, these ticks were shipped to Bundeswehr Institute of Microbiology, Munich, Germany, where they were reexamined and documented using a Keyence VHX-900F microscope (Itasca, IL). Images were then compared with specimens housed in the Gertrud Theiler Tick Museum – ARC-OVR in Pretoria, South Africa. The accession codes for the specimens used for comparison are as follows: *A. lepdium*—OP2573i, OP2573ii, OP2573iii, OP2617i, OP2617ii; *R. decoloratus*—OP2649i, OP2649ii, OP2685i, OP2685ii.

Animal owners or caretakers were interviewed using a structured questionnaire delivered electronically on a tablet. Questions covered topics such as knowledge about tick-borne diseases, use of protective measures against ticks and tick-borne diseases, and interactions with wildlife. The research project has been ethically approved by School of Veterinary Medicine and Animal Resources Research Ethics Committee, Makerere University, Uganda (SVARREC/03/2017) and Uganda National Council for Science and Technology, Uganda (A580). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in the studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. Animal owners/caretakers interviewed in the study have read, understood and signed an informed consent form. The three described specimens with anomalies have been deposited at Uganda Virus Research Institute Tick Museum, Entebbe, Uganda.

Results

In total, 7,521 ticks were collected from Moroto, Soroti, Gulu, Hoima, and Kasese districts of Uganda, from September to November 2017. Of the collected ticks, 2,594 were *Amblyomma* spp., 4,888 *Rhipicephalus* spp. and 39 *Hyalomma* spp. Three ticks showing morphological abnormalities included two *A. lepidum* females collected from Moroto district and one *R. decoloratus* female from Kasese district. These represented 0.04% of the total collected ticks (0.08% of the collected *Amblyomma* spp. and 0.02% of the collected *Rhipicephalus* spp.) and more specifically 0.28% (2/709) of the *A. lepidum* and 0.75% (1/134) of *R. decoloratus*. All specimens were obtained from cattle whose owners were pastoralists, who practice communal grazing and treat their cattle with acaricides by hand spraying. The two *A. lepidum* were collected from different animals on 10 October 2017 and 11 October 2017 in Kedepo village of Pupu parish, Rupa sub-county in Moroto district (GPS coordinates: 2.56424N and 34.62096E), while the *R. decoloratus* was collected on 26 September 2017 in Kijjungu village of Nyamugasani parish, Nyakatonzi sub-county in Kasese district (GPS coordinates: 0.04215S, 29.83603E).

General Anomaly

One *A. lepidum* specimen displayed gynandromorphism with the presence of both male and female features. It was the size of a female measuring 4.187 mm long from scapula to the end of festoon on the right side. This side had no anomalies. The left side measured 4.346 mm and had elements of male conscutum embedded in the female alloscutum dorsally. The specimen measured 3.383 mm wide (behind the third pair of legs). Small metallic, gold-crimson with greenish border patch ornamentations were embedded in the left side of the female alloscutum (Fig. 1A). This is characteristic of male ornamentation. Furthermore, the left side with embedded pieces of male conscutum was larger than the right side, with slight asymmetry and a clear delimitation of the female and male parts. The foveal glands were shifted. The basis capitulum showed female features ventrally and dorsally. In ventral view, the tick displayed general female characters, but with slight asymmetry on the right side. Additionally, the left stigma (spiracle) was slightly larger than the right (Fig. 1B). The coxae and genital aperture were typical of female and the hypostome presented denticles and other characters typical of females.

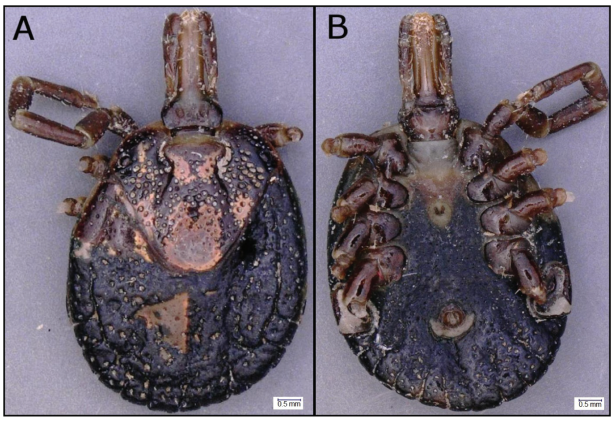

Fig. 1. Dorsal (A) and ventral (B) views of *Amblyomma lepidum* displaying gynandromorphism.

Local Anomaly

The second *A. lepidum* female, showed slight asymmetry on the left side (Fig. 2A) as well as absence of the genital aperture with both posterior lips and folds missing (Fig. 2B). It was the size of an adult *A. lepidum* with a total body length of 7.55 mm and width of 4.58 mm across the third coxa. The specimen did not show other morphological abnormalities.

Multiple Anomalies

One *R. decoloratus* female, almost completely engorged, displayed a slight asymmetry on its right side and multiple anomalies on its left side (Fig. 3A and B). The first leg on the left was completely missing (ectromely), while legs II, III, and IV on the same side were missing some segments. Leg II had only the coxa and trochanter, while legs III and IV had three segments; coxa, trochanter, and femur. Moreover, the femur of leg IV was atrophied. The anterior of coxa II and posterior of coxa IV showed chitinous formations. The cuticula displayed unusual folds due to the ectromely and presence of chitinous formations and other small constrictions on the idiosoma ventrally (Fig. 3B).

Discussion

General Anomalies

This is the first report of gynandromorphism in an *A. lepidum* collected from cattle. According to the classification system for tick gynandromorphs (Campana-Rouget 1959b), this specimen is considered to be a metagynander intriqué gynandromorph. Its morphology featured components of male characters embedded in areas of the female alloscutum dorsally, which shifted the position of the foveal glands. Shifted foveal glands are also rare in ticks and are mainly associated with gynandromorphism as previously observed in *Hyalomma detritum damascenium* (currently *Hyalomma scupense*) (Campana-Rouget 1959b, Apanaskevich et al. 2010).

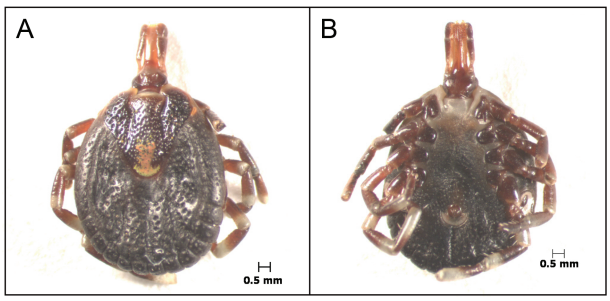

Fig. 2. Dorsal (A) and ventral (B) views of *Amblyomma lepidum* with absence of genital aperture.

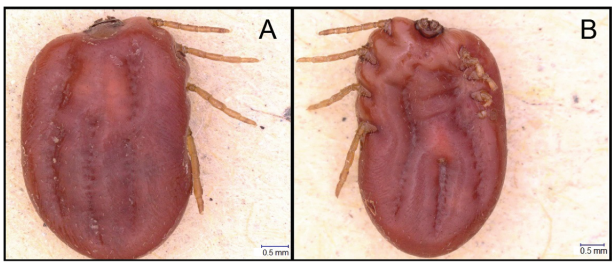

Fig. 3. Dorsal (A) and ventral (B) views of *Rhipicephalus decoloratus* with multiple morphological anomalies.

Gynandromorphism is a rare event in which both female and male phenotypic characteristics are simultaneously displayed in an organism (Campana-Rouget 1959b, Guglielmone et al. 1999, Keskin et al. 2012, Keskin et al. 2016). Despite growing trend in medico-veterinary research on ticks (Moyer 2015, Laroche et al. 2017), to date, approximately 81 natural cases of gynandromorphic ticks have been documented in Ixodidae (Labruna et al. 2000, Labruna et al. 2002, Keskin et al. 2012, Kar et al. 2015, Prusinski et al. 2015, Keskin et al. 2016, Chitimia-Dobler et al. 2017, Muñoz-Leal et al. 2018). Nevertheless, gynandromorphism is relatively frequent in natural populations of *Amblyomma* ticks, as compared with other genera (Labruna et al. 2002, Keskin et al. 2012, Keskin et al. 2016, Muñoz-Leal et al. 2018). The specimen we describe here was one in thousands of ticks we collected, representing 0.01% of the total and 0.04% of *Amblyomma* spp., respectively. Interestingly, this range of gynandromorphs is approximately the same as found in tick surveys in the United States (Prusinski et al. 2015), Europe (Chitimia-Dobler and Pfeffer 2017), and South America (Guglielmone et al. 1999). On a species level, the specimen with morphological anomalies were found in 0.28% (2/709) of the *A. lepidum* and 0.75% (1/134) of *R. decoloratus*.

When considering data from many different authors, more than 40% of gynanders appear in *Amblyomma* species (Dias 1953, Rechav 1977, Vinabal et al. 1994, Guglielmone et al. 1999). Consequently, different gynandromorphous *Amblyomma* cases have been described, i.e., protogynandres in *Amblyomma cajennense* (Fonseca 1935), *Amblyomma dissimile* (Brumpt 1934), *Amblyomma neumanni* (Vinabal et al. 1994), *Amblyomma tholloni* (Campana-Rouget 1959b), *Amblyomma hebraeum* (Rechav 1977), and *Amblyomma variegatum* (Stampfli 1985); gynandromophic intriqués in *Amblyomma scutatum* (Schulze 1933). The most common gynandromorphism, bipartite protogynander, was described in *Amblyomma oblongoguttatum* (Labruna et al. 2000), *A. cajennense* (Labruna et al. 2002), *Amblyomma imitator* (Sundman 1965), and *A. neumanni* (Guglielmone et al. 1999).

There are several hypotheses proposed to explain gynandromorphism in various organisms. The origin of gynandromorphism in ticks seems to result from eggs starting development as females, with the maternal sex chromosome (X) eliminated in the cleavage cells of the zygote (Pereira and Castro 1945, Oliver and Delfin 1967). This is similar to what was demonstrated in *Drosophila* flies (Sturtevant 1929). However, gynandromorphs may result from different types of impaired development, including double fertilization, somatic crossover between chromosomes, partial fertilization and sex chromosome non-disjunction, but all of these explanations seem less likely than sex chromosome elimination (Oliver and Delfin 1967). We did not test any of these putative reasons by genetic means, as we opted to maintain the morphological integrity of the specimen.

Local Anomalies

In *A. lepidum* females, the genital aperture is 'V' shaped and situated between coxae II-III (Voltzit and Keirans 2003, Walker et al. 2014). It forms the external opening of the reproductive system. Generally, the absence of a genital aperture missing both posterior lips and folds appears extremely rare in adult ticks. It was observed in *Ixodes ricinus* where it was considered as gynandromorphism (Campana-Rouget 1959b). In another protogynander *A. dissimile*, a genital aperture was not visible, but a fragment of the oviduct was found after dissection (Brumpt 1934). A similar case was in a genital aperture atrophy associated with gynandromorphism in *Rhipicephalus sanguineus* (Campana-Rouget 1959b). To the contrary, we did not observe any elements of gynandromorphism in our described tick specimen.

Multiple Anomalies

Multiple anomalies in ticks were previously described (Chitimia-Dobler et al. 2017). Asymmetry is a general anomaly (Campana-Rouget 1959b), but it can be associated with many local anomalies. The *R. decoloratus* female we describe here showed asymmetry, ectromely, constriction and chitinous formations that were most likely embedded palp segments. Asymmetry that was associated with ectromely or missing stigma was found on *Ixodes ricinus* (Chitimia-Dobler et al. 2017). Chitinous formations were also observed in *Hyalomma asiaticum* (Delpy 1936), *Ornithodoros tartakovsky* (Pavlovsky 1940), *Hyalomma dromedarii* (Alfeief 1948) and *Ixodes ricinus* (Campana-Rouget 1959b). Constrictions have been reported in *Hyalomma marginatum* (Keskin et al. 2016), *A. cajennense* and *Amblyomma parvum* (Guglielmone et al. 1999).

In conclusion, this study describes the presence of various morphological abnormalities in a collection of ticks obtained from a natural environment in an African setting. We report for the first time the presence of gynandromorphism in *A. lepidum* collected from cattle. The gynandromorphous specimen was a metagynander. Additionally, we document the absence of a genital aperture in another *A. lepidum* specimen and the presence of multiple anomalies in a *R. decoloratus*. This study should stimulate wide-scale investigation of this phenomenon in tick populations across the continent.

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