

External morphological anomalies in ixodid ticks from Thrace, Turkey

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Abstract Of 18,667 ticks examined, 33 specimens from species identified as *Haema-physalis parva*, *Hyalomma marginatum*, *Hy. scupense*, *Rhipicephalus bursa* and *Rh. turanicus* were found to have external morphological anomalies. Anomalous *Ha. parva*, *Hy. scupence* and *Rh. turanicus* were reported in this study for the first time. General anomalies manifested as asymmetry and deformations of the idiosoma, whereas local anomalies occurred in legs, exoskeleton, spiracular, adanal, subanal and accessory plates, mouthparts and capitulum. With this study describing a gynandromorphic *Hy. marginatum*, the number of gynandromorphic tick cases has been raised to two in Turkey.

Keywords Ticks · Anomalies · Haemaphysalis · Hyalomma · Rhipicephalus · Thrace

Introduction

Described in many articles, morphological anomalies in ticks have been known since L.G. Neumann who was a professor at National Veterinary School of Toulouse, France, and was the first to publish some anomalous ixodid ticks in 1899, as reported by Feldman-Muhsam (1950). External morphological anomalies which are seen much more frequently in ixodid ticks are generally grouped in two categories: General and local anomalies. While the formerincludes asymmetry, and bifurcation of the idiosoma, dwarfism, gigantism and gynandromorphism, the latter is related tooligomely, atrophy (brachymely), symely, heterosymely, anisomely, schistomely, ectomely, polymelyand heteromorphose (heteromorphosis) of legs, deformities of certain mouthparts, Haller's sensory organ and

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anus structure, oligomely of the porose area and chelicera, anomaly in the form and course of the anal groove (Campana-Rouget 1959; Haarløv 1962; Buczek et al. 1991, 2004 Buczek 1995; Leonovich and Belozerov 2004; Dergousoff and Chilton 2007; Nowak-Chmura 2012). However, anomalies in the structure of internal organs also can be seen in ticks (Laptev 1963; Latif et al. 1988). The anomalies of taxonomically important structures, especially in gnathosoma may lead to taxonomic errors, and most identification mistakes result from local anomalies (Buczek 2000). Therefore, knowledge of morphological anomalies, especially local anomalies in ticks can help taxonomists to determine the species affiliation of anomalous ticks (Buczek 1995). Additionally, anomalous ticks can be more efficient vectors of tick-borne pathogens; hence this situation should be taken into account in human cases of tick bites (Alekseev et al. 2007; Nowak-Chmura 2012). This study aims to report new cases of morphological anomalies found in both natural and laboratory populations of some ixodid tick species collected from Thrace, the European part of Turkey.

Materials and methods

The tick specimens examined were collected from Thrace, the European part of Turkey in 2013. A total of 18,667 tick specimens included 444 nymphs and 18,223 adults. The latter formed natural population of the present study. 178 *Hy. marginatum* adults (87 males and 91 females) were collected from the ground by flagging, thus all hungry. Of 114 *Rh. bursa* nymphs, 37 were collected from the ears of goats, while the rest of the specimens from cows by hand picking method. Engorged nymphs of *Hy. scupense* and *Rh. bursa* were reared in the laboratory at constant temperature of 25 °C and 80–85 % relative humidity (RH) for development into adults which formed laboratory population of this study.

All specimens were identified to species level, using taxonomic keys in Walker et al. (2003) and Estrada-Peña et al. (2004). External morphology of the specimens was examined, and anomalous individuals were photographed using a stereomicroscope (Leica EZ4 HD). In addition, some anomalies were studied in detail through scanning electron microscopy (SEM) (Quanta FEG 250).

The anomalies observed were classified as general anomalies and local anomalies depending on the extent of the anomalies and their location, as proposed by Nowak-Chmura (2012). In order to describe various types of leg anomalies, the terminology of Makol and Łaydanowicz (2006) and Buczek (2000) was followed in that the following terms were used: atrophy (brachymely) = shortening of appendages; heteromorphosis = deformation of appendage segments and joints; oligomely = complete absence of appendages.

The specimens with anomalies have been deposited in the tick collection, Department of Biology, Namik Kemal University in Tekirdag, Turkey.

Results

The tick material studied comprised five species in three genera: *Ha. parva, Hy. marginatum, Hy. scupense, Rh. bursa* and *Rh. turanicus.* Of 18,667 ticks examined, all in the adult stage, 33 specimens in five species were found to be anomalous (0.18 %). Table 1 presents frequency of anomalies based on both species and gender.

Species	Number of examined specimens	Number of anomalous specimens	% anomalous specimens
Ha. parva	208 (90 F, 118 M)	2 F	0.962 F
Hy. marginatum	11,677 (3521 F, 8156 M)	9 (5 F, 3 M, 1 G)	0.077 (0.142 F, 0.037 M, 0.009 G)
Hy. scupense	2998 (1424 F, 1574 M)	10 (5 F, 5 M)	0.334 (0.351 F, 0.318 M)
Rh. bursa	847 (362 F, 485 M)	4 (2 F, 2 M)	0.472 (0.553 F, 0.412 M)
Rh. turanicus	2937 (1467 F, 1470 M)	8 (4 F, 4 M)	0.272 (0.273 F, 0.272 M)
Total	18,667 (6864 F, 11,803 M)	33 (18 F, 14 M, 1G))	0.177 (0.262 F, 0.119 M)

Table 1 Frequency of anomalies in tick species studied

F female, M male, G gynandromorphic

In the laboratory population including 444 adults which were developed from the nymphs, three specimens displayed anomaly (0.68 %). Frequency of anomalies in natural populations of ticks was 0.17 %. Of 178 unfed *Hy. marginatum* adults, four specimens were anomalous (2.25 %).

General anomalies manifested as asymmetry and deformations of the idiosoma, whereas local anomalies occurred in legs, exoskeleton, spiracular, adanal, subanal and accessory plates, mouthparts and capitulum. All anomalies recognized and described are summarized in Table 2.

One *Hy. marginatum* displayed gynandromorphism, the presence of both male and female features within the same organism. This gynandromorphic specimen was characterized by male features in dorsal view, whereas it lacked exact adanal plates, subanal plate and accessory plate. Those plates were in the appearance of protuberances without sclerization. Furthermore, mouthparts of this specimen were turned ventrally from the basis capituli (Fig. 3).

Due to overlap of various general and local anomalies at the same time, a total of 48 anomalous characters were described in 33 anomalous specimens. Of these, 34 characters (70.8 %) were related to local anomalies, and the rest to general anomalies. Exoskeleton anomalies were predominant within local anomalies, followed in decreasing order by spiracular plate and leg anomalies (23.5 and 20.6 %, respectively). As to general anomalies, nine anomalies out of 14 manifested as asymmetry of the idiosoma (64.3 %).

Discussion

Several tick species have been reported to have anomalous morphological characteristics to date, including *Amblyomma cajennense* (Labruna et al. 2002), *A. flavomaculatum* (Nowak-Chmura 2012), *A. latum* (Nowak-Chmura 2012), *A. oblongoguttatum* (Labruna et al. 2000), *A. variegatum* (Latif et al. 1988), *Argas arboreus* (Belozerov et al. 2003), *A. persicus* (Buczek et al. 1991), *A. polonicus* (Siuda 1981), *A. radiatus* (Obenchain and Oliver 1972), *A. reflexus* (Buczek 1988, 1991, 1993; Buczek et al. 2004), *Boophilus annulatus* (Sakla et al. 1980; Caeiro and Simões 1990), *B. australis* and *B. decoloratus* (Nuttall 1914), *Dermacentor andersoni* (Dergousoff and Chilton 2007), *D. marginatus* (Caeiro and Simões 1990), *D. occidentalis* (Oliver and Delfin 1967), *D. reticulatus*

Species	No. and gender	Description of anomalies	
Ha. parva	1 Female	Deformed scutum	
	1 Female	Asymmetry of the idiosoma marked in the wrinkled rear	
Hy. marginatum	1 Female	Asymmetry of the idiosoma marked by distortion on its left rear side lacking spiracular plates, anus being moved to left side and crinkled body surface	
	1 Female	Atrophy of the first and second left and the third right legs	
	1 Female	Atrophy of the fourth right leg	
	1 Female	atrophy of the second right and the fourth left leg appendage segments of which are longer than their counterparts	
	1 Female	Oligomely of the second and fourth left legs and atrophy of the third left leg (Fig. 1)	
	1 Male	Asymmetry of the idiosoma lacking spiracular plate on left side, wrinkled and undulated consucutum (Fig. 2)	
	1 Male	Deformations in the rear edge of the idiosoma	
	1 Male	Oligomely of the third left leg and atrophy of the fourth left leg	
	1 Gyn	Rudimentary plates on a wrinkled area and mouthparts turned ventrally from basis capituli (Fig. 3)	
Hy. scupense	1 Female	atrophy of all legs, the first right leg strongly reduced and with deformed segments (Fig. 4)	
	1 Female	Asymmetry of the idiosoma marked in the crinkled rear	
	1 Female	Asymmetry of the idiosoma deformed in the rear	
	1 Female	Asymmetry of the idiosoma and wrinkled scutum	
	1 Female	Markedly wrinkled scutum	
	1 Male	Rudimentary anal, adanal and postanal plates on the right side in which there is a second rudimentary spiracular plate next to the spiracular plate (Fig. 5)	
	1 Male	Dented and deformed consucutum	
	1 Male	Asymmetry of the idiosoma, and wrinkled and dented conscutum	
	1 Male	Rudimentary palps (Fig. 6)	
	1 Male	Deformed idiosoma	
Rh. bursa	1 Female	Lack of capitulum, heteromorphose of the first three legs on both sides and atrophy of both fourth legs (Fig. 7)	
	1 Female	Asymmetry of the idiosoma marked by an indentation on the left side at the level of the fourth leg	
	1 Male	Asymmetry of the idiosoma, and deformed adanal plates	
	1 Male	Distorted adanal plate	
Rh. turanicus	1 Female	Deformation in the rear of scutum	
	1 Female	Rudimentary spiracular plate	
	1 Female 2 Males	Spiracular plate with bifurcated tail (Fig. 8)	
	1 Female 2 Males	Spiracular plate with trifurcated tail	

Table 2 Anomalies recognized in tick species studied

No. number of specimens, Gyn gynandromorphic

Fig. 1 Hyalomma marginatum, female, oligomely of the second and fourth left legs and atrophy of the third left leg





Fig. 2 Hyalomma marginatum, male, wrinkled and undulated consucutum and asymmetry of the idiosoma, lacking spiracular plate on left side



Fig. 3 Gynandromorphic *Hyalomma marginatum* with mouthparts turned ventrally from the basis capituli, and lacking exact adanal plates, subanal plate and accessory plate which were in the appearance of protuberances without sclerization



Fig. 4 Hyalomma scupense, female, strongly reduced first right leg with deformed segments



Fig. 5 *Hyalomma scupense*, male, rudimentary anal, adanal and postanal plates on the *right side* in which there is a second rudimentary spiracular plate next to the spiracular plate

Fig. 6 *Hyalomma scupense*, male with rudimentary palps



(Alekseev et al. 2008; Buczek et al. 2013), *Haemaphysalis neumanni* (Laptev 1963), *H. punctata* and *H. sulcata* (Tovornik 1987), *H. longicornis* and *H. turturis* (Belozerov and Leonovich 1995), *Hyalomma aegyptium* (Nuttall 1914; Nowak-Chmura 2012), *H. asiaticum* (Leonovich and Belozerov 2004), *H. lusitanicum* (Caeiro and Simões 1990), *H.*



Fig. 7 *Rhipicephalus bursa*, female lacking capitulum and with heteromorphose of the first three legs on both sides and with atrophy of both fourth legs



Fig. 8 Spiracular plate with bifurcated tail observed in one female and two males Rhipicephalus turanicus

marginatum (Caeiro and Simões 1990; Buczek et al. 1991; Buczek 2000; Buczek et al. 2004; Keskin et al. 2012), *H. savignyi* (Feldman-Muhsam 1950), *Ixodes arboricola* (Haarløv 1962), *I. hexagonus* (Tovornik 1987), *I. persulcatus* (Alekseev et al. 2000; Zharkov et al. 2000; Alekseev et al. 2007), *I. ricinus* (Tovornik 1987; Buczek et al. 1991; Zharkov et al. 2000; Belozerov 2003; Alekseev et al. 2008; Žygutienė et al. 2008), *I. scapularis* (Kittler 2011), *I. trianguliceps* (Tovornik 1987), *Ornithodorus moubata* (Robinson 1944), *Rhipicephalus bursa* (Caeiro and Simões 1990) and *R. sanguineus* (Nuttall 1914; Caeiro and Simões 1990; Estrada-Peña 2001; Labruna et al. 2002).

Anomalous *Ha. parva*, *Hy. scupence* and *Rh. turanicus* were reported in this study for the first time. However, morphological anomalies are not only seen in ticks, but also in mites (Madej et al. 2004; Makol and Łaydanowicz 2006) and some other arthropods

including the beetle *Tenebrio molitor* (Balazuc 1948), the millipede *Glomeris marginata* (Juberthie-Jupeau 1969), the centipede *Stigmatogaster subterranean* (Leśniewska et al. 2009) and the spider *Tegenaria atrica* (Templin and Napiórkowska 2013).

Morphological anomalies which can be met at any developmental stage rarely occur in natural populations of ixodid ticks, with frequencies ranging from about 0.03 % to about 0.62 %. The percentage of anomalous specimens studied is concordant with the results of other researchers (Tovornik 1987; Latif et al. 1988; Buczek 1995; Guglielmone et al. 1999; Nowak-Chmura 2012). Environmental factors such as temperature, RH (Siuda 1981; Buczek 1988, 1991, 2000), pollution (Zharkov et al. 2000; Kittler 2011), and chemical agents including insecticides (Robinson 1944; Buczek et al. 2013), retinoic acid (Belozerov 2003, 2004), iodine compounds (Buczek 1993), sulfuric acid and ethyl quinone (Campana-Rouget 1959) were reported to lead to anomalies in both natural and experimental populations. Moreover, host resistance to tick infestation is known to change the morphology of subsequent tick development stages (Estrada-Peña 2001). Depending on pollution levels, ticks may exhibit higher rate of morphological anomalies (6-48 % of ticks in different populations) in polluted environments (Zharkov et al. 2000; Kittler 2011). Frequencies of anomalies observed in both natural and laboratory populations of this study are in accordance with the previous studies. Anomalies observed herein cannot be ascribed to an environmental factor such as pollution and/or chemical agents, since sampling areas were villages and their vicinity which are far away from industrial regions, and in which agriculture is restricted to basic needs, thus excluding pollution and pesticides as environmental factors responsible for anomalies. However, high temperature and RH can be effective causes, since sampling villages are hot (average temperature 27 °C) and receive high precipitation in summers, whereas winters are cold (average temperature 4 °C) and rainy (Gargili et al. 2010). Apart from environmental factors, genetic mutations, interspecies hybrids, regeneration after injury or damage to the body, density during parasitization of the host, parasitizing non-typical and accidental hosts, and lack of food are proposed as the reasons for the formation of anomalies in natural populations of ticks (Nowak-Chmura 2012).

In ixodid ticks, there are numerous reported cases of leg (Sakla et al. 1980; Tovornik 1987; Buczek et al.1991; Buczek 1995; Estrada-Peña 2001; Dergousoff and Chilton 2007; Nowak-Chmura 2012) and exoskeleton (Zharkov et al. 2000; Alekseev et al. 2007; Žygutienė et al. 2008; Kittler 2011) anomalies, and asymmetry of the idiosoma (Tovornik 1987; Latif et al. 1988; Buczek et al. 1991; Siuda et al. 2006; Nowak-Chmura 2012), while information on anomalies of spiracular, adanal, subanal and accessory plates, capitulum and mouthparts is rare. It is reported that abnormal embryonic development, adverse moulting conditions, injuries, abnormal regeneration and breeding under conditions of high humidity are the causes of leg anomalies in ticks, and in some cases these anomalies may be hereditary. On the other hand, the loss of legs, which causes the idiosoma to bend towards where there are no legs, loosening of the tissues and abnormal regeneration during metamorphosis from the immature stages to the adult, or the underdevelopment of the structures and organs in the idiosoma were suggested as the reasons for asymmetry of the idiosoma. Of the leading anomalies in this study, spiracular plate anomalies which may hinder the determination of the species and even the genus of a tick were also observed in many ticks of the genera Hyalomma, Rhipicephalus and Amblyomma (Nowak-Chmura 2012).

Gynandromorphism in ticks is an extraordinarily rare event in nature. To date, some 61 natural cases of gynandromorphic ticks have been reported from *Amblyomma* (17 cases), *Dermacentor* (2), *Haemaphysalis* (2), *Hyalomma* (21), *Ixodes* (5), and *Rhipicephalus* (14), the frequency of gynandromorphism in species of *Amblyomma* and *Hyalomma* being

relatively higher in nature (Labruna et al. 2002; Keskin et al. 2012). Although various anomalies in ixodid ticks were documented in Turkey, one case of gynandromorphism has been reported in *Hy. marginatum* to date (Keskin et al. 2012). With this study, the number of gynandromorphic tick cases has been raised to two in Turkey.

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