Ticks and Tick-borne Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Ticks (Acari: Ixodidae) of Nepal: First record of *Amblyomma varanense* (Supino), with an update of species list

Shyam Kumar Pun[a], Alberto A. Guglielmone[b,*], Evelina L. Tarragona[b], Santiago Nava[b], Mahendra Maharjan[a]

[a] Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
[b] Instituto Nacional de Tecnología Agropecuaria and Consejo Nacional de Investigaciones Científicas y Técnica, Estación Experimental Agropecuaria Rafaela, ruta 34 km 227, 2300, Rafaela, Santa Fe, Argentina

ARTICLE INFO

Keywords:
Nepal
Orthriophis hodgsoni
Amblyomma varanense
Sub-genus *Aponomma*
Ixodidae
Species list
Hosts

ABSTRACT

Males and females specimens of *Amblyomma* were collected from *Orthriophis hodgsoni* (Squamata: Colubridae) caught during routine herpetological work in Kathmandu. Morphological characteristics led to the diagnosis of *A. varanense*, constituting the second Nepalese species for the genus after the collection of *Amblyomma gervaisi*, also from a colubrid snake, almost 100 years ago. *Amblyomma varanense* is the 55th species found in the country, and preceded for the collection of 21 species of *Haemaphysalis*, 14 *Ixodes*, 6 *Rhipicephalus*, 6 *Hyalomma*, 4 *Dermacentor*, 1 *Amblyomma*, 1 *Anomalohimalaya* and 1 species of *Nosomma*. Eleven and 19 species have been found on humans and domestic mammals, respectively, evidencing the medical and economic importance of Nepalese Ixodidae.

1. Introduction

Nepal is a Himalayan country where the Oriental and Palearctic realms extensively converge. Its peculiar biogeography contains an ample fauna of hard ticks where eight of the 12 extant genera of Ixodidae are represented, namely: *Amblyomma*, *Anomalohimalaya*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Nosomma* and *Rhipicephalus*. The genus *Amblyomma* is formed by 138 species worldwide (Guglielmone et al., 2015), but its presence in Nepal is unusual, with one record of a male of *Amblyomma gervaisi*, named as *Aponomma gervaisi*, collected from *Ptyas mucosa* (named as *Zamenis mucosus*) listed in Sharif (1928), and one specimen of an undetermined *Amblyomma* found on a cow in Banke District by Bohara and Shrestha (2015). Conversely, the remainder seven genera are relatively well represented in the country, but a compendium of the Nepalese species of Ixodidae is not available, with the exception of the genus *Ixodes*, that was revised by Clifford et al. (1975).

Males and females of *Amblyomma varanense*, a new species for the ixodid fauna of Nepal previously referred to as *Amblyomma* sp. in Pun and Maharjan (2016), have been collected and details of this record are presented thereafter. Alongside this new species record, an updated list of the Ixodidae of Nepal is provided believing that this summary should be of value for workers interested in ticks in general and Asian species in particular.

2. Materials and methods

2.1. Tick collection and morphological identification

Four females with different degrees of engorgement, and eight male ticks were collected by the senior author from an *Orthriophis hodgsoni* (Squamata: Colubridae), named as *Elaphe hodgsoni* in the study of Pun and Maharjan (2016), captured during a routine herpetological survey on May 18, 2013 at Chi-ba-hāh (27°41′N 85°17′E), Kirtipur, Kathmandu Valley, located in Eastern Himalayas in the Oriental Zoogeographic Region (Olson et al., 2001). Tick specimens were preserved in 70% ethanol and cleared by both light and scanning electron microscopy (SEM) with ultrasound (20 kHz) using distilled water and commercial detergent in a proportion of 9:1. All specimens were measured to be compared with the information provided by Kaufman (1972, see below) for males (length from apices of the scapulae to posterior body margin and wide) and females (length of the scutum from apices of the scapulae to posterior scutal margin and wide). Measures are in mm, and presented as average followed by the range. The best preserved specimens were photographed using a Nikon Alphaphot-2 YSZ optical microscope. SEMs were taken at the Servicio de Microscopía Electrónica, Museo de

* Corresponding author at: Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, ruta 34 km 227, 2300, Rafaela, Santa Fe, Argentina.
E-mail addresses: rauyan.pun2@gmail.com (S.K. Pun), guglielmone.alberto@inta.gob.ar (A.A. Guglielmone), tarragona.evelina@inta.gob.ar (E.L. Tarragona), nava.santiago@inta.gob.ar (S. Nava), mmaharjan@cdztu.edu.np (M. Maharjan).

https://doi.org/10.1016/j.ttbdis.2018.01.010
Received 16 November 2017; Received in revised form 13 January 2018; Accepted 20 January 2018

La Plata, Universidad Nacional de La Plata, Argentina, using a JEOL/JSM 6360 LV® Digital Scanning Microscope. Two failed attempts were made to obtain DNA samples from male and female ticks; therefore, valuable information for molecular taxonomy is not available. The specimens are preserved in the INTA Rafaela Tick Collection under the accession number 2376.

Ticks were identified to genus level using the key of Durden and Beati (2014). The combination of anal groove surrounding the anus posteriorly, eyeless specimens, festoons at posterior body margin, subcylindrical elongate palps, trochanters without spurs, and absence of ventral plates indicates that all specimens belong to the genus *Amblyomma* subgenus *Aponomma* Therefore, the descriptions and keys for the former genus *Aponomma* of Kaufman (1972) were used to reach a specific diagnosis. The study of Kaufman (1972) contains species that are actually included in the genus *Bothriocroton* as defined in Klompen et al. (2002), but also taxa with poor support for their inclusion in the subgenus *Aponomma* as shown in Miller et al. (2007), Burger et al. (2012, 2013), Durden and Beati (2014), Guglielmone and Nava (2014) and Williams-Newkirk et al. (2015), namely: the Nearctic non-*Amblyomma elaphense*, the Australasian non-*Amblyomma sphenodonti*, the Afrotropical non-*Amblyomma transversale* and its closely related species or, eventually its synonym, non-*Amblyomma orlovi*. In brief, the subgenus *Aponomma* is treated here as formed by 15 species, the Afrotropical *Amblyomma arcanum*, *A. exornatum*, *A. flavomaculatum*, *A. inopinatum*, *A. latum*, the Afrotropical-Oriental *A. gervaisi*, the Australasian *A. komodoense*, *A. kraneveldi*, *A. soembabwense*, the Australasian-Oriental species *A. fimbriatum*, *A. trimaculatum*, *A. varanense* and the Oriental taxa *A. crassipes*, *A. fuscolineatum* and *A. pattoni*, all prone to parasitize squamatan hosts (Guglielmone et al., 2014).

2.2. List of species of Ixodidae from Nepal

A non-extensive literature review was carried out to construct a list of hard ticks, and their hosts, in Nepalese lands, with comments when appropriate. Whenever possible, scientific names of wild hosts have been updated to current nomenclature. Class, orders and families of hosts are presented in Table 1.

3. Results

3.1. Tick diagnosis and relevant morphological characters

3.1.1. Diagnosis

Male ticks are characterized by hypostomal dentition 3/3; coxa I with two spurs, the external longer than the internal; scutum with five spots of iridescent ornamentation, none located in the scapular area. The hypostomal dentition and spurring of coxa I in female ticks equal as in males; female scutum with three spots of iridescent ornamentation, none located in the scapular area, with a few small to large punctations. These morphological features are congruent with the description of *A. varanense*.

3.1.2. Relevant morphological characters of the male (Figs. 1A–E; 3A)

Body outline subcircular, almost as long as broad, broadest posteriorly at level of spiracular plates; length from apices of hypostome to posterior body margin: 3.14 (3.00–3.33); length from apices of scapulae to posterior body margin: 2.43 (2.34–2.64); wide at level of spiracular plates: 2.53 (2.40–2.67). *Scutum*: eyes absent; reddish-brown with five yellowish-green-whitish spots, one central broadly circular, two irregular posterior spots and two lateral elongate spots; few and small punctations scattered over the central and anterolateral fields, few and large punctations on the posterolateral fields; marginal groove absent; festoons broader than long; cervical grooves short, deep, comma shaped. *Venter*: bearing rather numerous setae, longer on the posterior field; genital opening at level of coxa II; anus at level of spiracular plate, surrounded posteriorly by a marked anal groove; spiracular plate sub-

Table 1
Class, order and family of hosts for Nepalese ixodid ticks.

MAMMALIA
ARTIODACTYLA[a]: BOVIDAE
Cattle
Domestic buffalo
Domestic yak
Dzo (hybrids of cattle and yaks)
Goat
Sheep
Antilope cervicapra
Boselaphus tragocamelus
Capricornis thar
Hemitragus jemlahicus
Naemorhedus goral
Pseudois nayaur
ARTIODACTYLA: CERVIDAE
Axis axis
Axis porcinus
Cervus elaphus
Muntiacus muntjak
Rusa unicolor
ARTIODACTYLA: MOSCHIDAE
Moschus sp.
ARTIODACTYLA: SUIDAE
Domestic pig
Wild pig
CARNIVORA: AILURIDAE
Ailurus fulgens
CARNIVORA: CANIDAE
Dog
Canis aureus
Cuon alpinus
Vulpes bengalensis
CARNIVORA: FELIDAE
Domestic cat
Felis sp.
Felis chaus
Panthera pardus
Panthera tigris
Prionailurus bengalensis
Prionailurus viverrinus
CARNIVORA: HERPESTIDAE
Herpestes auropunctatus
Herpestes edwardsii
CARNIVORA: MUSTELIDAE
Martes flavigula
Mustela sibirica
CARNIVORA: URSIDAE
Ursus thibetanus
CARNIVORA: VIVERRIDAE
Paguma larvata
Paradoxurus hermaphroditus
Viverra zibetha
CHIROPTERA: PTEROPODIDAE
Cynopterus sp.
LAGOMORPHA: LEPORIDAE
Lepus nigricollis
Lepus oiostulus
Ochotona sp.
Ochotona roylei
EQUIDAE
Horse
PERISSODACTYLA: RHINOCEROTIDAE
Rhinocerus unicornis
PRIMATES: HOMINIDAE
Human
RODENTIA: CRICETIDAE
Alticola sp.
Alticola stoliczkanus
Neodon sikimensis
Phaiomys leucurus
RODENTIA: MURIDAE
Apodemus sp.
Apodemus gurkha
Bandicota sp.
Golunda ellioti
Millardia meltada

(*continued on next page*)