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Original article

The geographic distribution of *Argas* (*Persicargas*) *miniatus* and *Argas* (*Persicargas*) *persicus* (Acari: Argasidae) in America, with morphological and molecular diagnoses from Brazil, Chile and Cuba

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ABSTRACT

High similarity of morphological traits has historically overshadowed the identities and distributions of poultry-associated soft ticks *Argas* (*Persicargas*) *miniatus* and *Argas* (*Persicargas*) *persicus* in America. In order to model the occurrence of both parasites in the continent, in the current study we performed morphological and molecular analyses to identify ticks collected in hen houses from Brazil and northern Chile. Combining these results with literature data, and the examination of *Argas* allotments deposited in the tick collections "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" (Brazil), the "Coleção Acarológica do Instituto Butantan São Paulo" (Brazil), and the "Colección Zoológica de la Academia de Ciencia de Cuba" (Cuba), we present a critical list with the localities where *A.* (*P.*) *miniatus* and *A.* (*P.*) *persicus* have been reported in the American continent. Our results confirmed the presence of *A.* (*P.*) *miniatus* in Brazil and Cuba, and *A.* (*P.*) *persicus* in Chile, which in particular, constitutes the first molecularly confirmed report of the later species for South America. Although *A.* (*P.*) *miniatus* and *A.* (*P.*) *persicus* have been documented in 21 American countries, the identity of some reports must still be considered as uncertain until detailed morphological and/or molecular studies are performed. When contrasted to a Köppen-Geiger climate classification, *A.* (*P.*) *miniatus* predominantly occurs in equatorial and *A.* (*P.*) *persicus* in arid climates. However, until undetermined reports of both species are correctly identified, any conclusion on their geo-climatological occurrence throughout the American continent would be rather speculative.

1. Introduction

Ticks of the genus *Argas* Latreille (Argasidae) are haematophagous parasites in all their postembryonic stages and are currently represented by 61 species distributed in all the Zoogeographic Regions of the world (Guglielmone et al., 2010). Based on a morphological approach of immature and mature stages, taxonomic summaries of this genus have proposed to divide most of its specific diversity in six defined subgenera, namely *Argas*, *Carios*, *Chiropterargas*, *Microargas*, *Persicargas, Secretargas* and an undefined subgenus referring to *Argas bureschi* Dryenski 1957 (Hoogstraal, 1985). Particularly, the *Argas* (*Persicargas*) group is composed by 16 ornithophilous species phenotypically similar to each other (Hoogstraal, 1985; Estrada-Peña et al., 2003), and well adapted to parasitize domestic birds (Hoogstraal, 1956;

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Kohls et al., 1970), to which they can transmit pathogenic eubacteria (Burroughs, 1947; Zaher et al., 1977; Lisbôa et al., 2009) and viruses (Hoogstraal, 1985).

Argas (*Persicargas*) *miniatus* was insufficiently described by Koch (1844) from post-larval stages collected in Guyana (Demerara region), and its identity remained problematical until Kohls et al. (1970) reexamined the type specimens. Based on the anatomy of the peripheral integumental cells of two damaged specimens, these authors concluded that *A.* (*P.*) *miniatus* was a valid taxon, and redescribed the species from previous treatments as a morphological variety (i.e. var. *miniatus* and var. *dissimile*) (Neumann, 1904; Aragão, 1938) and a synonym of *A.* (*P.*) *persicus* (Nuttall et al., 1908). In that same work, by the examination of several collections of immature and mature *Argas* from American localities, the authors presented the distribution of *A.* (*P.*) *miniatus,* including Brazil, Colombia, Guyana, Panama and Trinidad and Tobago (Kohls et al., 1970). The last morphologically confirmed report of this tick species was made from larvae, nymphs and adults collected in Rio Grande do Sul State in Brazil (Evans et al., 2000). Yet, *A.* (*P.*) *miniatus* has been also reported from Venezuela (Vogelsang and Dias, 1953), Cuba (De La Cruz, 1974), Puerto Rico (Capriles and Gaud, 1977), Jamaica and the United States (Keirans, 1984).

The tick *A.* (*P.*) *persicus* was described by Oken (1818) from poultry-associated specimens collected in Iran, yet subsequent reports put in evidence a transcontinental distribution, almost always in association to domestic chicken (Hoogstraal, 1956). To date, *A.* (*P.*) *persicus* has documented reports in all the Zoogeographic Regions of the world with the exception of Antarctica, including the majority of African countries (Hoogstraal, 1956; Cumming, 1999), Australia (Petney et al., 2004), China (Chen et al., 2010), Italy (Pantaleoni et al., 2010) and India (Keirans, 1984). In America, morphological studies have identified this tick from specimens collected in the United Sates, Paraguay (Kohls et al., 1970) and Argentina (Nava et al., 2004). Still uncertain are the reports from Cuba (De La Cruz 1976) and particularly from Chile, where a morphological variety named as *A.* (*P.*) *persicus* var. *porteri* and *A.* (*P.*) *persicus* were documented from the Metropolitan Region and Calama, respectively (Lahille, 1915; Porter, 1928). Both Chilean records were subsequently considered as possible misidentifications with *Argas* (*Argas*) *neghmei* Kohls & Hoogstraal 1961 (Kohls and Hoogstraal, 1961).

The study of the larval phenotype is crucial in order to separate species in the Argasidae family (Hoogstraal, 1985; Klompen, 1992), and might constitute a suitable approach to report new or confirm doubtful records for *A.* (*P.*) *miniatus* and *A.* (*P.*) *persicus* in America. While the morphology of nymphs and adult stages has shown to be less informative, some features of the dorsal integument constitute useful discrete characters for a specific diagnosis in the *Argas* genus. In order to confirm the identity of *Argas* ticks associated with domestic chicken, in this study we combine morphological and molecular analyses of immature and mature specimens collected in chicken houses from several states of Brazil, the North of Chile and Cuba. Additionally, we present a map with the current distribution and the identity status of the analyzed species in America.

2. Material and methods

2.1. Examined material

Two nymphs, six males and one female collected in Calama (22°27′S; 68°54′W, elevation 2775 m), Antofagasta Region, Chile, date of collection 03 December 2014; 16 larvae, 41 nymphs, 25 females and 22 males collected in Santa Teresinha (07°05′S; 37°26′W; elevation 303 m), Paraiba State, Brazil, date of collection April 2014; and 13 nymphs, 11 females, and five males collected in Brasília (15°37′S; 47°56′W, elevation 1250 m), Distrito Federal, Brazil, date of collection 23 October 2016. All these collections of immature and mature soft ticks were made during daytime inside chicken houses by examining fissures in wooden and concrete-made structures. Subsequently, ticks were transported alive to the laboratory and engorged females were placed in an incubator with 25 °C and 80% of relative humidity in order to obtain ovipositions. In addition to this field-collected material, we examined *Argas* allotments deposited in the three following tick collections: Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva (CNC), Brazil: CNC-878 (two females, four males), CNC-1153 (ten larvae, seven nymphs, seven females, six males), CNC-1154 (five nymphs, ten females, ten males), and CNC-2576 (one nymph, three males, two females); Coleção Acarológica do Instituto Butantan, São Paulo (IBSP), Brazil: IBSP-731 (five nymphs, four females, one male), IBSP-803 (two females, one male), IBSP-863 (one female, two males), IBSP-937 (two nymphs, 12 females, ten males), IBSP-1267 (two nymphs, four females), IBSP-1269 (26 females, ten males), IBSP-1279 (seven females, two males), IBSP-1746 (two females, seven males), IBSP-4214 (seven nymphs, six females, five males), IBSP-4317 (28 nymphs, 18 females, 12 males), IBSP-4513 (one nymph, ten females, one male), IBSP-5954 (two males), IBSP-9251 (two females, one male), and IBSP-9934 (one nymph, two males); and the Colección Zoológica de la Academia de Ciencia de Cuba (CZACC), Cuba: N°10704 (one nymph, nine females, six males), and N°10734 (four nymphs, seven females, eight males).

2.2. Morphological analyses

Two cohorts of ten laboratory-reared larvae, each one obtained from a unique female per locality, and ten larvae, 12 females and one male from the CNC were clarified in a 20% KOH solution, and mounted in slides using Hoyer's medium. In order to visualize the setal pattern with detail and to compare with the original description of other Argasinae, the capitulum of adult specimens was mounted apart from the idiosoma, following the dissection methodology proposed by Cooley and Kohls (1944). Morphological characters of slide-mounted larvae (Fig. 1) and adults were observed under light microscopy and measured above micrographs using the software Image-Plus Pro v5.1. Post-larval specimens from the CNC, CZACC and IBSP were examined using a SteREO Discovery V12 stereomicroscope and measured with the software ZEN 2 pro. All measurements are given in millimeters with the standard deviation followed by the range in parenthesis. We adopted morphological definitions of Sonenshine et al. (1962) for larvae of *Argas* genus, which consider that only the subgenus *Chiropterargas* has dorsolateral setae arranged in an anterior and posterior groups. As a clear anatomical definition of both groups of setae is not specified either for *A.* (*P.*) *miniatus* or *A.* (*P.*) *persicus*, we did not consider these characters in our study and rather obtained averages of dorsolateral setae.

In order to discriminate the relationship between morphologically similar species, obtained measures of unengorged larvae were submitted to a principal component analysis (PCA) based on Pearson correlation matrix for 40 morphological variables (Table 1). Measurements of *Argas* (*Persicargas*) *keiransi* Estrada-Peña, Venzal & González-Acuña 2003 were also included in the comparisons.

To observe in detail morphological characters with specific significance, one female of the CZACC was prepared for electron microscopy photographs following Corwin et al. (1979). Subgenus level determination using larval stages followed Kaiser et al. (1964). Species level identification were performed by comparing the obtained morphological and morphometrical data with original descriptions (Kohls and Hoogstraal, 1961; Estrada-Peña et al., 2003) and redescriptions of Argasinae from America (Kohls et al., 1970).

2.3. Molecular tools

To confirm morphological diagnoses, DNA extraction using the Guanidine Isothiocyanate technique (Sangioni et al., 2005) was individually performed in three females, three nymphs and two larvae from Calama (Chile), one male and two larvae from Santa Teresinha